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The Tubulin Code, from Molecules to Health and Disease

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Abstract

Microtubules are essential dynamic polymers composed of α/β-tubulin heterodimers. They support intracellular trafficking, cell division, cellular motility, and other essential cellular processes. In many species, both α-tubulin and β-tubulin are encoded by multiple genes with distinct expression profiles and functionality. Microtubules are further diversified through abundant posttranslational modifications, which are added and removed by a suite of enzymes to form complex, stereotyped cellular arrays. The genetic and chemical diversity of tubulin constitute a tubulin code that regulates intrinsic microtubule properties and is read by cellular effectors, such as molecular motors and microtubule-associated proteins, to provide spatial and temporal specificity to microtubules in cells. In this review, we synthesize the rapidly expanding tubulin code literature and highlight limitations and opportunities for the field. As complex microtubule arrays underlie essential physiological processes, a better understanding of how cells employ the tubulin code has important implications for human disease ranging from cancer to neurological disorders.

Contents

1. INTRODUCTION

Microtubules are cytoskeletal polymers essential for myriad cellular functions, including cytoarchitecture, intracellular transport, ciliogenesis, cell migration, and cell division. Their underlying subunit, the α/β-tubulin heterodimer, stacks longitudinally to form polar protofilaments that then associate laterally into the hollow, cylindrical microtubule (**Figure 1*a***). Microtubules undergo polymerization and depolymerization phases, termed dynamic instability (Mitchison & Kirschner 1984, Horio & Hotani 1986), and yet they support both highly active and long-lived structures. They form the barrel-like structure of the mitotic spindle for rapid chromosomal orchestration, the column of an axoneme built to withstand large mechanical forces or relay environmental cues, and the scaffold of an axon that extends and pathfinds to generate stable and reactive circuits. Since early on, the apparent compositional and structural homogeneity of the microtubule polymer has been at odds with such remarkably varied architectures and activities across cell types, tissues, and organisms.

1.1. Development of the Tubulin Code Hypothesis

Fulton & Simpson (1976) noticed that the dramatic morphological transformation of the unicellular *Naegleria* from mitotic amoeba to flagellate was accompanied by an abrupt appearance of antigenically unique tubulin proteins (see also Fulton 2022). This observation led them to propose the "multi-tubulin hypothesis," which posits that multiple tubulin isoforms exist within an organism and can be differentially expressed and possess nonredundant properties.

We now know that the seemingly simple *Naegleria* has 13 α- and 9 β-tubulins; two of each are expressed exclusively in mitosis (Velle et al. 2022). Indeed, comparative sequence analysis (Cleveland et al. 1980) and now whole-genome sequencing have revealed that many organisms possess a suite of α- and β-tubulin genes, termed isoforms (not to be confused with RNA splice isoforms, which derive from the same gene). The number varies widely across organisms (e.g., *Chlamydomonas reinhardtii* has one α and one β gene, *Tetrahymena thermophila* three α and six

(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

The multiple layers of the tubulin code, from isoforms and posttranslational modifications to cellular effectors. (*a*) Genetic diversity. Organisms express multiple α- and β-tubulins. (*b*) Chemical diversity. Tubulin isoforms are functionalized with posttranslational modifications added or removed by enzymes referred to as tubulin code writers and erasers. These modifications include acetylation (Ac; *light blue*), methylation (Me; *dark blue*), phosphorylation (P; *yellow*), glycylation (G; *gray*), glutamylation (E; *fuchsia*), and tyrosination (Y; *brown*). The dotted line delineates initiases (*top*) versus elongases (*bottom*). The tubulin code is read out by different classes of readers: (*c*) structural MAPs and (*d*) additional effectors, such as molecular motors. The impact of each modification based on in vitro reconstitution studies is summarized in the table where an upward-facing arrow, downward-facing arrow, and dash refer to stimulation, inhibition, and no change, respectively, while empty cells indicate a lack of in vitro studies with well-defined microtubule species. Note that microtubule tails and effectors are not drawn to scale, and the distribution of modifications, MAPs, and effectors is displayed across a single microtubule for ease of depiction only. (*e*) Tubulin isoforms and modifications regulate intrinsic microtubule properties such as structure, dynamics, and mechanics, and, in combination with the diverse cast of readers, regulate microtubule dynamics, organization, and cellular activities. Abbreviations: MAP, microtubule-associated protein; pf, protofilament; +TIPs, plus-end tracking proteins. Figure adapted from image created in BioRender.

β, *Saccharomyces cerevisiae* two α and one β, *Caenorhabditis elegans* nine α and six β, *Drosophila melanogaster* five α and five β, and *Mus musculus* seven α and eight β). Humans possess nine α- and nine β-tubulin genes (**Figures 1*a*** and **2**). Some isoforms differ by only a few residues, such as TUBA1A and TUBA1B, while others are highly divergent. Sequence analysis shows that the structural core/GTP-binding region of α- and β-tubulins is strongly conserved (97% and 95%, respectively), consistent with the need to retain the GTPase activity key to the polymerization/depolymerization cycle. Instead, variability lies at (*a*) polymerization interfaces, either lateral (between neighboring protofilaments) or longitudinal (between tubulin dimers along the same protofilament), or (*b*) the last 15 residues of the disordered and negatively charged (glutamate-rich) C-terminal tails (Roll-Mecak 2020). The C-terminal tails are the sites of the largest sequence variation. These tails project from the microtubule lattice (**Figure 1*a***) and lie at the primary interface for interaction with molecular effectors, termed readers, such as motor proteins and microtubule-associated proteins (MAPs) (**Figure 1*c*,*d***).

The C-terminal tails are also the dominant sites of extensive posttranslational modifications, such as detyrosination, glutamylation, glycylation, and phosphorylation, which—in addition to modifications at other tubulin interfaces, like phosphorylation, acetylation, methylation, palmitoylation, sumoylation, and polyamination—serve as another major source of microtubule diversification (**Figure 1*b***). Tubulin modifications are added and removed by specialized enzymes, termed writers and erasers, which show substrate preference for either the microtubule polymer or the soluble tubulin dimer, and act combinatorially to form complex and stereotyped modification patterns. These layers of genetic and chemical diversification together constitute a tubulin code that modifies the intrinsic properties of microtubules and is read out by cellular effectors (**Figure 1*c*,*d***) to achieve the functional heterogeneity of the cellular microtubule array (Roll-Mecak 2020). In this review, we highlight recent advances in our understanding of the molecular and cellular mechanisms of the tubulin code and the many fundamental questions that remain.

1.2. The Tubulin Life Cycle: Global Regulation, Incorporation, and Turnover

α- and β-tubulin mRNAs, once transcribed from various genomic loci, are translated and folded into assembly-competent α/β-tubulin heterodimers by tubulin chaperones (Tian & Cowan 2013). Ultimately, the microtubules in any given cell exist as a "mosaic" (Roll-Mecak 2019) composed of different α- and β-tubulins, with polymer representation governed by their stoichiometry and their incorporation rates into the microtubule (**Figure 1*a***). Accumulating evidence supporting localized translation in polarized cell types (Das et al. 2021) raises the possibility that mRNA transport mediates differential subcellular sorting of tubulin isoforms into specialized microtubules. *TUBA1A*

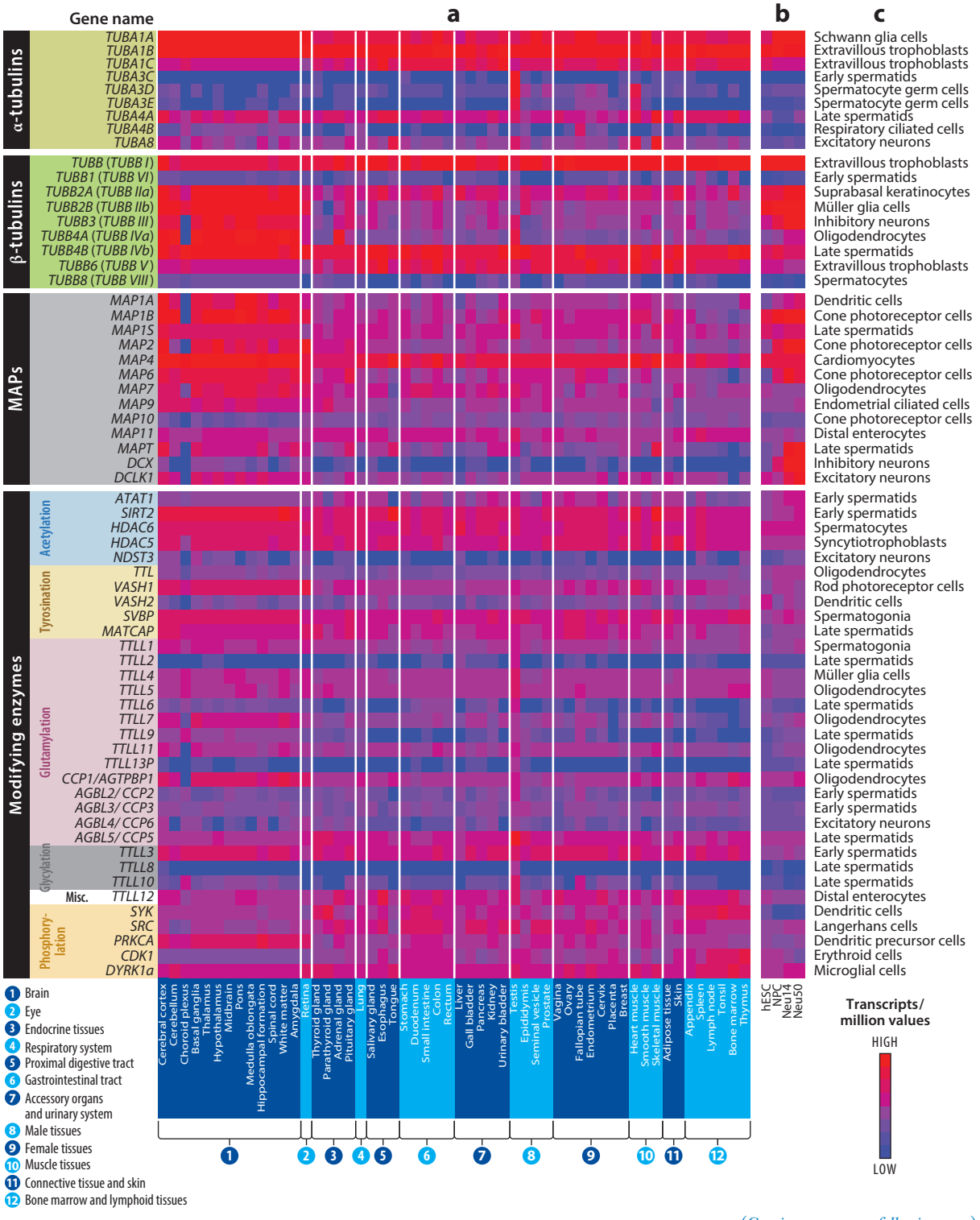

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Figure 2 (*Figure appears on preceding page*)

Spatiotemporal expression of tubulin isoforms, MAPs, and modifying enzymes. (*a*) Human tissue expression (obtained from RNA consensus tissue gene data from the Human Protein Atlas and compiled from Human Protein Atlas and Genotype–Tissue Expression transcriptomics data) plotted as a heat map of the log-transformed normalized transcripts per million values. (Normalization for the heat-map range was performed independently for each gene category: α-tubulins, β-tubulins, MAPs, and modifying enzymes.) (*b*) RNA levels over the course of differentiation from hESCs to NPCs to neurons at day 14 (Neu14) and day 50 (Neu50) (Blair et al. 2017). (*c*) Top-expressing cell type for each gene (based on RNA single-cell-type data from the Human Protein Atlas). Abbreviations: hESCs, human embryonic stem cells; MAPs, microtubule-associated proteins; NPCs, neuronal progenitor cells.

and *TUBB2B* transcripts, for example, have been identified in distal axonal growth cones (Zivraj et al. 2010, Gumy et al. 2011, Preitner et al. 2014).

As microtubules are self-assembling polymers, their dynamics and abundance are directly tied to α/β-tubulin subunit concentrations (Mitchison & Kirschner 1984). Therefore, one initial hypothesis was that tubulin gene expansion was driven primarily by tissue- or development-specific demands for microtubule mass. Cells possess a highly conserved mechanism, termed tubulin autoregulation, to monitor the soluble tubulin pool, which integrates tubulin transcription, translation, sequestration by free-tubulin binding proteins, microtubule incorporation, and tubulin turnover. If cellular soluble tubulin levels get too high (such as upon destabilization of microtubules) or too low, the cell buffers this effect by degrading or stabilizing tubulin transcripts, respectively (Cleveland et al. 1981). This process occurs cotranslationally via recognition of the β-tubulin N terminus by tetratricopeptide repeat domain 5, which binds to the first four N-terminal residues as they emerge from the ribosome to recruit RNA decay factors to tubulin-translating ribosomes (Yen et al. 1988, Lin et al. 2020). As all β-tubulin isoforms share this four residue N-terminal motif, this mechanism coordinates the abundance of every isoform simultaneously. This feedback mechanism must be carefully considered when interpreting phenotypes associated with mutation or manipulation of individual isoforms, as loss of one tubulin isoform can result in compensatory changes in the abundance of others.

2. GENETIC DIVERSITY

The first concrete evidence for the multitubulin hypothesis emerged in 1982 in studies of spermatogenesis in flies (Kemphues et al. 1982). Effective spermatogenesis requires coordination of multiple microtubule-based structures, including the meiotic spindle and motile cilia, that use the dominant testis-specific β2 isoform. In a foundational experiment, Hoyle & Raff (1990) swapped the β2 isoform with β3 and found that these assemblies were lost. They narrowed this functional difference to the C-terminal tail—partly restoring axoneme structure by swapping the β2 tail onto β3 (Hoyle et al. 1995). Since that time, phenotypic screens, functional studies, in vitro research, and human genetic studies have reinforced the hypothesis that individual isoforms play nonredundant roles in regulating intrinsic microtubule properties and are integrated by modifying enzymes, MAPs, and motors to fine-tune microtubule-mediated processes (**Figure 1*e***). We highlight several classic and emerging examples below.

2.1. Tubulin Isoforms Regulate Microtubule Structure and Dynamics

Specific tubulin isoforms can govern microtubule assembly and dynamics. In an early example, a *C. elegans* phenotype screen yielded touch-insensitive mutants that mapped to genes encoding mec-12 (Fukushige et al. 1999) and mec-7 (Savage et al. 1989), α- and β-tubulin isoforms, respectively. These isoforms support 15 protofilament microtubule assemblies, present in subsets of touch-receptor neurons and distinct from the 11 protofilament microtubules characteristic of *C. elegans*. Isoform-dependent microtubule organization is also found in platelets. Here, the

marginal band of microtubules gives platelets their discoid shape and requires expression of the most divergent (78% homology) β-tubulin isoform TUBB1 (Wang et al. 1986). Loss of this isoform results in marginal band disorganization (Schwer et al. 2001, Kunishima et al. 2009). In cancer cell lines, overexpression of the ubiquitous, albeit weakly expressed, TUBB6 led to sparser, fragmented microtubule arrays, suggesting that this isoform destabilizes microtubules (Bhattacharya & Cabral 2004).

Until very recently, it was unclear whether changes observed in vivo in microtubule structure and dynamics were due to *cis* effects of the tubulin isoform alone or *trans* effects mediated by factors that recognize these isoforms. While early studies with tubulin isolated from different tissues or species hinted at intrinsic isoform-dependent differences (Banerjee et al. 1990, Lu & Luduena 1994, Panda et al. 1994), a clear mechanistic understanding of the contributions of individual isoforms was not possible because biochemical experiments in the field were performed with tubulin purified from brain tissue. Unfortunately, brain tubulin, while highly abundant, is highly heterogeneous, containing multiple tubulin isoforms and posttranslational modifications (Vemu et al. 2017). Moreover, brain tubulin isoform distribution is very different from that of most other cell types in the human body (**Figure 2**). The recent ability to affinity-purify tubulin from various sources (Widlund et al. 2012) and to generate recombinant mammalian tubulin (Minoura et al. 2013, Vemu et al. 2016, Ti et al. 2018) now permits isoform evaluation outside cellular complexity and enables identification of the specific residues conferring these properties (Pamula et al. 2016, Vemu et al. 2016). In vitro single-microtubule dynamics assays using recombinant, isotypically pure tubulin showed that TUBB3 microtubules catastrophe (i.e., switch from polymerization to depolymerization) more frequently than TUBB2B microtubules (Pamula et al. 2016), and titrated addition of TUBB3 to an isoform composition found in HEK293 cells (predominantly TUBA1A, TUBB, and TUBB4B) proportionally increased catastrophe and reduced the microtubule growth rate (Vemu et al. 2017). Tubulin purified from HEK293 cells has lower critical concentration, faster growth, and lower catastrophe rates compared with brain tubulin (Vemu et al. 2017), highlighting the differences between tubulins from different sources.

The increased dynamicity of microtubules conferred by TUBB3 in vitro aligns with results from in vivo studies. TUBB3 knockout mice with no gross differences in neuroanatomy or behavior display defects in regenerative response to peripheral nerve injury with decreased microtubule growth cone dynamics and reduced neurite outgrowth (Latremoliere et al. 2018). Intrinsic differences in tubulin dynamics also help control spindle size, with a striking correlation between microtubule growth and stability and larger or smaller spindles for *Xenopus laevis* and *Xenopus tropicalis*, respectively (Hirst et al. 2020). Recent research has shown that α-tubulin isoforms can also regulate dynamics. Recombinant TUBA1C/TUBB2A mouse microtubules grow faster and catastrophe less compared with TUBA1A/TUBB2B microtubules, and these differences are mediated by residues in the C-terminal tail of TUBA1A alone (Diao et al. 2021).

2.2. Tubulin Isoform Readers

Tubulin isoforms can also control the construction of specialized assemblies through differential recruitment of readers. TUB1 and TUB3, the two β-tubulin isoforms in budding yeast, were presumed interchangeable because TUB3 overexpression upon TUB1 knockout rescues lethality (Schatz et al. 1986). However, single-isoform strains wherein each gene was swapped into the endogenous locus of the other showed that the isoforms mediate distinct mitotic spindle positioning mechanisms, likely through differential recruitment of dynein versus KAR9 (Nsamba et al. 2021). Recent proteomics studies revealed isoform-specific interactors. TUBB6 expression is high in osteoclasts, which mediate bone resorption through actin-rich podosomes. TUBB6

knockout reduces microtubule growth rates, results in defective/immature podosomes, and reduces resorption (Maurin et al. 2021), which may be tied to decreased association with Rho GTPase–activating protein 10, a CDC42 regulator, and lymphocyte-specific protein 1, an actin regulator (Maurin et al. 2021). In prostate cancers, TUBB4A interaction with myosin-9 (MYH9) is thought to facilitate cell migration by protecting the nucleus upon passage through constricted regions through crosstalk with the actin cytoskeleton (Gao et al. 2022). Studies linking TUBB3 to mitochondrial organization (with TUBB3 knockdown causing increased fragmentation and decreased mitochondrial dynamics) identified TUBB3-specific interactions with Parkinson disease protein 7 and tropomyosin 3A, regulators of mitochondrial and actin dynamics, respectively (Parker et al. 2022). Taken together, these studies support direct or indirect roles for specific tubulin isoforms in cytoskeletal crosstalk and organelle organization.

2.3. Tubulin Isoforms in Disease

A diverse set of human pathologies caused by mutations in tubulin isoforms, termed tubulinopathies, highlight tissues, developmental stages, and structures where specific isoforms are most relevant (**Figure 2*a–c***) and expose nonredundant functions (for reviews, see Romaniello et al. 2018, Hoff et al. 2022, Maillard et al. 2023). The broad spectrum of disease phenotypes associated with tubulin isoforms reflects (*a*) the relative ratios of isoforms, (*b*) the compensatory capacity of remaining isoforms, and (*c*) whether the mutation fully removes the isoform, fails to incorporate or incorporates with dominant-negative effects, and/or alters specific modification or interaction sites. Rare cases of female infertility due to oocyte meiotic arrest traced to *TUBB8* mutations identified TUBB8 as the dominant isoform in oocytes and early embryos (Feng et al. 2016). TUBB6 is upregulated during human primary muscle cell differentiation and in regenerating muscle fibers when microtubules display a longitudinal arrangement that precedes the mature lattice-like network characteristic of mature skeletal muscle fibers (Randazzo et al. 2019). These findings appear to be connected to Duchenne's muscular dystrophy, where persistent TUBB6 upregulation underlies microtubule disorganization and where reducing TUBB6 restores microtubule directionality (Randazzo et al. 2019). TUBB4A/B have long been associated with ciliary structures (Jensen-Smith et al. 2003, Vent et al. 2005). However, the intersection of ciliopathies with tubulinopathies emerged only recently from the discovery of de novo *TUBB4B* mutations in patients with primary ciliary dyskinesia (Mechaussier et al. 2022). TUBB4B is expressed specifically in early ciliogenesis, and patient-derived respiratory epithelial cells harboring TUBB4B mutations display fewer basal bodies and disorganized, stunted, or absent axonemes.

Most strikingly, tubulinopathies reinforce the significance of tubulin diversity in the mammalian nervous system (Blair et al. 2017) (**Figure 2*b***). Mutations in *TUBA1A* (Hebebrand et al. 2019), *TUBA8*, *TUBB* (Breuss et al. 2012), *TUBB2A* (Cushion et al. 2014, Sferra et al. 2018), *TUBB2B* (Jaglin et al. 2009, Cushion et al. 2014), *TUBB3* (Poirier et al. 2010, Tischfield et al. 2010, Saillour et al. 2014), and *TUBB4A* (Simons et al. 2013, Sase et al. 2020) are associated with neurodevelopmental or neurodegenerative disease. Mutation of *TUBB3*, for example, the neuron-specific β-tubulin isoform (**Figure 2*b***), results in ocular motility disorder, hypoplasia in the ocular motor nerve, and Charcot-Marie-Tooth type 2–like sensorimotor polyneuropathy (Tischfield et al. 2010).

Shifts in tubulin isoform ratios are common across tumorigenesis and in response to chemotherapies (often microtubule-targeting agents) (for reviews, see Prassanawar & Panda 2019, Lopes & Maiato 2020). High levels of TUBB4A are tied to aggressive prostate cancers (Gao et al. 2022), while lower TUBB4B levels coincide with increased migration of metastatic colon cancer (Sobierajska et al. 2019). The normally brain-specific TUBB3 is dramatically increased in breast

and lung cancers (Leandro-García et al. 2010) and is associated with increased metastatic potential. Increased TUBB3 levels also emerge in drug-resistant tumors, and elevated TUBB3 expression is a prognostic marker for poor responsiveness to taxanes (Bartolomeo et al. 2020). Elevated TUBB6, the destabilizing β-tubulin isoform, is prognostic for glioblastomas (Jiang et al. 2020) yet is also associated with prolonged responsiveness and survival in non-small-cell lung cancers in response to taxane-based chemotherapies (Verdier-Pinard et al. 2003, Christoph et al. 2012). Characterizing the isoform composition of tumors is therefore critical for determining prognosis and treatment.

In this section, we have shown how a dynamic and mosaic polymer composed of varied tubulin isoforms can drive unique functionality. However, it is merely the core scaffold upon which all subsequent microtubule functionalization takes place through additional diversification, explored more fully below.

3. CHEMICAL DIVERSITY

Tubulin posttranslational modifications further increase microtubule array complexity, enabling rapid, reversible microtubule alterations. The most heavily modified microtubules first described were axonemal microtubules found in cilia and flagella, and these have been the source of foundational insights into the chemical diversity of tubulin (Guichard et al. 2023). Recent discovery of the enzymes responsible for writing and erasing tubulin modifications and newly developed biochemical platforms for recombinant tubulin and in vitro reconstitution of the tubulin code (Roll-Mecak 2020) have enabled investigations into the kinetics and substrate preferences of many modification enzymes, as well as downstream effects on molecular readers, such as MAPs and motors. These studies have shed light on how the tubulin code is written, maintained, and integrated across cellular pathways and tissues.

3.1. Detyrosination

Detyrosination involves the reversible removal of the terminal tyrosine from α-tubulin.

3.1.1. Writers/erasers. Most α-tubulin isoforms genetically encode a C-terminal tyrosine, which is catalytically removed and ligated as part of a detyrosination/tyrosination cycle. The vasohibins VASH1 and VASH2, in complex with essential cofactor small vasohibin-binding protein (SVBP) (Aillaud et al. 2017, Nieuwenhuis et al. 2017), were the first enzymes discovered to detyrosinate microtubules (**Figure 1**). VASH1 and VASH2 exhibit different dynamics, generating distinct modification patterns in vitro: diffuse for VASH1 and punctate for VASH2 (Ramirez-Rios et al. 2023). Recently, microtubule-associated tyrosine carboxypeptidase (MATCAP) was identified as a third detyrosinase (Landskron et al. 2022). MATCAP is a metalloproteinase, not a cysteine protease like VASH1/2. Combined SVBP and MATCAP knockout in mice abolished brain tubulin detyrosination, indicating that VASH1/2 and MATCAP enzymes are responsible for most, if not all, detyrosination (Landskron et al. 2022). Several X-ray and cryo-electron microscopy (cryo-EM) studies elucidated the structures of all three enzymes, shedding light on their mechanisms of substrate recognition (Adamopoulos et al. 2019; Li et al. 2019, 2020; Wang et al. 2019; Landskron et al. 2022).

Tyrosination, the religation of the C-terminal tyrosine, is catalyzed by tubulin tyrosine ligase (TTL) (Raybin & Flavin 1975). The structure of TTL revealed a catalytic core common to the ATP-GRASP superfamily enzymes, whose members catalyze the reaction of a carboxylic acid with a nucleophile (Szyk et al. 2011). In the case of TTL, the nucleophile is tyrosine. This ancient structural scaffold is shared among all tubulin modification enzymes that ligate amino acids (tyrosine, glutamate, and glycine). Functional and structural studies have shown that TTL modifies the

tubulin dimer (Raybin & Flavin 1975, Szyk et al. 2011, Prota et al. 2013), while VASH1/2 modify the microtubule polymer (Aillaud et al. 2017, Li et al. 2020). Thus, detyrosination accumulates with microtubule lifetime. Upon depolymerization, detyrosinated tubulin is quickly retyrosinated on the basis of TTL cellular concentration and catalytic rate (Szyk et al. 2011).

Detyrosinated α-tubulin can be further modified into Δ2- and Δ3-tubulin, found mainly in neurons, by cytosolic carboxypeptidases (CCPs) (Paturle-Lafanechère et al. 1991, 1994; Aillaud et al. 2016). Δ2- and Δ3-tubulin are not TTL substrates and, therefore, are thought to be irreversibly modified (Paturle-Lafanechère et al. 1991).

3.1.2. Direct effects on microtubule properties. Tyrosination and detyrosination are associated with dynamicity and stability of cellular microtubules, respectively (Kreis 1987, Schulze & Kirschner 1987, Webster et al. 1987). However, a recent study using recombinant, engineered human tubulins demonstrated definitively that tyrosinated and detyrosinated microtubules have similar intrinsic dynamic parameters and that the differential cellular stabilities observed for these two microtubule subpopulations are due exclusively to effector recruitment (Chen et al. 2021). Δ2-tubulin, associated with stable cellular microtubules, also has dynamic parameters indistinguishable from those of tyrosinated tubulin (Chen et al. 2021), indicating that this stability also results from effector interactions.

3.1.3. Readers. Tyrosination recruits plus-end tracking proteins (+TIPs), which accumulate on growing microtubule ends. The +TIP complex includes ending-binding (EB) proteins and CAP-Gly domain–containing proteins, such as CLIP-170, among others. EB1–microtubule binding is not sensitive to tyrosination (Chen et al. 2021), but CLIP-170 recruitment is, because CAP-Gly domains specifically recognize the C-terminal EEY/F sequence (Peris et al. 2006, Weisbrich et al. 2007, Bieling et al. 2008) (**Figure 1*d***). In vitro reconstitution with recombinant human tyrosinated and detyrosinated tubulin showed that EB1 and CLIP-170 synergize on tyrosinated microtubules to increase growth rates and catastrophe frequencies (i.e., increasing microtubule dynamicity) (Chen et al. 2021). Tyrosination also increases recruitment of depolymerizing motor kinesin-13, contributing to the increased turnover of this microtubule subpopulation (Peris et al. 2009) (**Figure 1**). The severing enzyme katanin also prefers tyrosinated microtubules by recognizing the tyrosinated α-tail through its accessory, noncatalytic microtubule binding domains (Szczesna et al. 2022) (**Figure 1**). Thus, tyrosination-dependent effector recruitment increases microtubule growth rates and turnover, and its absence renders detyrosinated microtubules less dynamic. A recent proteomics study characterized the repertoire of tyrosination-sensitive readers (Hotta et al. 2022) in HeLa cells and found echinoderm-MAP-like 2, a factor that preferentially binds tyrosinated microtubules, localizing to depolymerizing microtubule plus ends and enhancing their chance of rescue (the switch from depolymerization to growth) (Hotta et al. 2022). Similar screens in the context of other modification enzymes in cell types enriched in particular modifications will likely advance the known repertoire of modification-dependent readers and identify cell type–specific readers.

Multiple motors are also sensitive to detyrosination/tyrosination (**Figure 1*d***). Tyrosination controls the loading of the dynein/BICD2/dynactin complex onto microtubules for transport initiation (McKenney et al. 2016, Nirschl et al. 2016) (**Figure 3**). Kinesin-2 (KIF17) is more processive on detyrosinated microtubules (Sirajuddin et al. 2014). Likewise, the kinetochore-associated motor CENP-E binds better and is more processive on detyrosinated microtubules, guiding chromosome alignment during mitosis (Barisic et al. 2015) (**Figure 3**). Optical trap force measurements also showed that CENP-E is able to withstand larger loads on detyrosinated microtubules (Barisic et al. 2015). Interestingly, kinesin-1 (KIF5B) motility is slightly reduced by detyrosination in vitro (Sirajuddin et al. 2014), though kinesin-1 (KIF5C) binding and

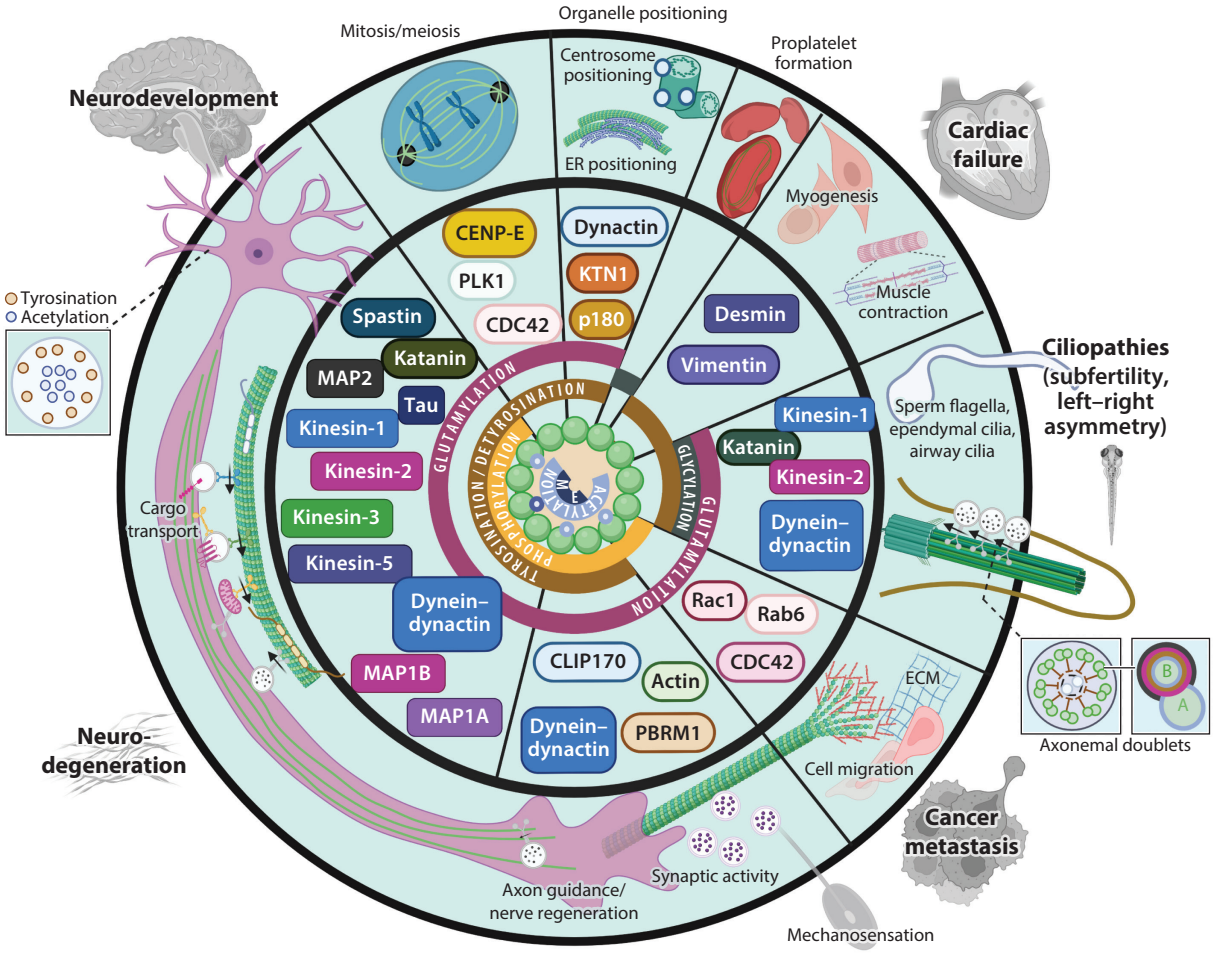

Figure 3

Tubulin code regulation of molecular effectors, cellular pathways, and disease pathogenesis. Schematic of a microtubule with concentric circles denoting the posttranslational modifications acetylation (*light blue*), methylation (*dark blue*), phosphorylation (*yellow*), glycylation (*dark green*), glutamylation (*fuchsia*), and detyrosination/tyrosination (*brown*). Tubulin posttranslational modifications regulate molecular effectors (*black inner ring*) and contribute to intracellular transport, cell migration, cilia function, myogenesis and muscle contractility, proplatelet formation, organelle positioning, cell division, neuronal arborization, and axon outgrowth (*black outer ring*). Diseases associated with misregulation of tubulin modifications are shown in gray. Abbreviations: ECM, extracellular matrix; ER, endoplasmic reticulum; ME, methylation. Figure adapted from image created in BioRender.

localization correlate with detyrosinated microtubules in cells (Dunn et al. 2008, Cai et al. 2009). This observation suggests an indirect effect, either through other tubulin modifications coinciding with detyrosination or through recruitment of effectors.

Some tubulin isoforms bypass the tyrosination cycle. For example, TUBA8, expressed in a narrow window of cortical neurogenesis and critical for differentiation of a specific intermediate progenitor subpopulation, encodes a C-terminal phenylalanine instead of tyrosine, making it impervious to detyrosination and subsequent Δ2-tubulin modifications (Ramos et al. 2020). Thus, TUBA8 incorporation commits microtubules toward high dynamicity through permanent recruitment of +TIP factors (Chen et al. 2021). TUBA8 mutants with either a TUBA1A tail swap

or addition of the C-terminal tyrosine overexpressed in mice fail to drive neurogenesis (Ramos et al. 2020). Conversely, TUBA4A reflects a genetically encoded detyrosinated state, characterized by a lack of C-terminal tyrosine, though it is capable of enzymatic tyrosination (Cambray-Deakin & Burgoyne 1990). This isoform is critical in platelets for the organization of the marginal band, enriched in stable microtubules, wherein mutations cause macrothrombocytopenia in humans and mice (Strassel et al. 2019). TUBA4A expression increases in mature neurons rather than during development. The functional relevance of this naturally detyrosinated isoform in aged neurons is unknown; however, TUBA4A mutations that prevent incorporation into microtubules are associated with the neurodegenerative disease amyotrophic lateral sclerosis (Smith et al. 2014), and recent single-nucleus RNA sequencing found TUBA4A enriched in human motor neurons during maturation or aging (Yadav et al. 2023).

3.1.4. Cellular functions and disease. Cell division depends on the precise spatiotemporal distribution of tyrosinated and detyrosinated microtubules. Specifically, kinetochore motor CENP-E guides chromosome alignment during mitosis in a detyrosination-dependent manner (Barisic et al. 2015) (**Figure 3**), and tyrosination, enriched on astral microtubules, is important for mitotic spindle formation and positioning (Gundersen & Bulinski 1986, Peris et al. 2006) (**Figure 3**). In meiosis, CDC42 signaling at the cell cortex causes asymmetry of the meiotic spindle, with more detyrosination on the egg side allowing biased centromere orientation and asymmetric division of genetic elements (Akera et al. 2017) (**Figure 3**).

An early study showing modification-dependent effector recruitment identified detyrosination as critical for vimentin intermediate filament localization to microtubules (Kreitzer et al. 1999). More recent research in cardiomyocytes showed that desmin intermediate filament localization to microtubules requires detyrosination (Robison et al. 2016, Chen et al. 2018). The desmin–microtubule interaction regulates the microtubule buckling that opposes the actin–myosin force that generates muscle contraction. Blocking detyrosination impairs myogenesis (Chang et al. 2002), while elevated detyrosination impairs cardiomyocyte contractility (Robison et al. 2016) (**Figure 3**), which can be rescued through pharmacological and genetic reduction of detyrosination (Chen et al. 2018). In epithelial cells, lysosomes are enriched along detyrosinated microtubules in a kinesin-1-dependent manner where they exhibit reduced motility and increased encounters and fusion with autophagosomes. Reducing this subpopulation of detyrosinated microtubules results in decreased autolysosome formation upon cell starvation (Mohan et al. 2019).

In neurons, tyrosination ensures efficient loading of dynein–dynactin at presynaptic nerve terminals (**Figure 3**) (Nirschl et al. 2016), consistent with the stimulatory effect of tyrosination on dynein–dynactin microtubule binding in vitro (McKenney et al. 2016, Nirschl et al. 2016). The recruitment of dynein to tyrosinated microtubules, mediated through the dynactin CAP-Gly domain, also controls centrosome positioning (Barbosa et al. 2017) (**Figure 3**). VASH1/2 double knockout in mice reduces detyrosination and disrupts neurogenesis, resulting in structural brain abnormalities (Aillaud et al. 2017) (**Figure 3**). Similarly, SVBP knockout mice, also detyrosination deficient, have microcephaly and other structural brain abnormalities (Pagnamenta et al. 2019). Autosomal recessive *SVBP* mutations in humans cause neurodevelopmental disorders, characterized by microcephaly and ataxia (Iqbal et al. 2019, Pagnamenta et al. 2019). TTL knockout in mice, on the other hand, is embryonic lethal (Erck et al. 2005), suggesting that detyrosination reversibility is essential. Subtle changes in TTL activity also modify organism fitness. *TTL* heterozygous mice exhibit Alzheimer's disease phenotypes, including loss of synaptic plasticity (Peris et al. 2022) (**Figure 3**), and TTL overexpression rescues Aβ peptide–induced pathologies in primary neurons, such as loss of microtubule invasion into dendritic spines and dendritic spine pruning (Peris et al. 2022).

3.2. Acetylation and Methylation

Acetylation and methylation involve the addition of an acetyl and methyl group, respectively, to lysine 40 in α-tubulin. Acetylation at other positions in tubulins is not covered in this section.

3.2.1. Writers/erasers. The first tubulin acetylation site identified was α-tubulin lysine 40 (K40) (L'Hernault & Rosenbaum 1985, LeDizet & Piperno 1987), a residue within a loop inside the microtubule lumen (L'Hernault & Rosenbaum 1985, Soppina et al. 2012, Nogales et al. 1999); in contrast, most tubulin modifications cluster on the C-terminal tails (**Figure 1**). K40 is acetylated by tubulin acetyltransferase (TAT) (Akella et al. 2010, Shida et al. 2010), a highly conserved enzyme, and removed by histone deacetylase 6 (HDAC6) (Hubbert et al. 2002) and sirtuin 2 (SIRT2) (North et al. 2003) (**Figure 1**). TAT has no known cellular substrates other than tubulin, whereas HDAC6 and SIRT2 have broad substrate profiles. For instance, HDAC6 also deacetylates HSP90 (Kovacs et al. 2005) and cortactin (Zhang et al. 2007). The TAT structure complexed with the acetylation loop elucidated the K40 recognition mechanism (Szyk et al. 2014). Despite needing access to the microtubule lumen, TAT prefers the microtubule polymer over the soluble tubulin dimer (Maruta et al. 1986, Akella et al. 2010, Kormendi et al. 2012) by recognizing features at the lateral protofilament interface (Szyk et al. 2014). HDAC6 prefers the tubulin dimer (Miyake et al. 2016, Skultetyova et al. 2017), while SIRT2 can modify both tubulin dimers and microtubules (North et al. 2003). This substrate specificity creates asymmetry such that acetylation accumulates on the microtubule polymer. Other tubulin acetylation sites (Choudhary et al. 2009) and deacetylation pathways (Cho & Cavalli 2012, Tang et al. 2021) exist, but their functional significance is not as well-studied as K40.

Tubulin is also methylated at K40 (Kearns et al. 2021), competing with acetylation at this site. The tubulin methyltransferase is histone methyltransferase set domain–containing 2 (SETD2), a component of the histone code (Park et al. 2016). Competition between acetylation and methylation is well described for histones (Latham & Dent 2007). In the case of histone 3 lysine 9, acetylation and methylation compete with opposing outcomes on transcriptional activity (Latham & Dent 2007). Interestingly, nucleosome-associated proteins are also glutamylated (Bachanova et al. 2015). Thus, multiple modification enzymes are shared between tubulin- and histone-associated proteins, possibly coordinating microtubule and chromosome dynamics. Importantly, in cellulo expression of SETD2 mutants revealed that distinct regions control histone and microtubule association, making it possible to discriminate SETD2 function within each pathway (Park et al. 2016, Kearns et al. 2021). Unlike TAT, SETD2 preferentially modifies tubulin dimers (Kearns et al. 2021). Thus, the balance of tubulin acetylation and methylation in cells is correlated with microtubule dynamics.

3.2.2. Direct effects on microtubule properties. Acetylation weakens lateral contacts between protofilaments and enhances the flexibility of microtubules, making them more resistant to mechanical deformation (Portran et al. 2017, Eshun-Wilson et al. 2019). Cellular microtubules lacking acetylation are frequently fragmented (Portran et al. 2017). It is interesting to speculate that K40 methylation may alter microtubule stability by blocking acetylation.

3.2.3. Readers. Cryo-EM reconstructions of *Chlamydomonas reinhardtii* axonemes and *Toxoplasma gondii* cortical microtubules revealed that numerous microtubule inner proteins (MIPs) bind the K40 loop and thus may read K40 acetylation directly (Ma et al. 2019, Wang et al. 2021). These MIPs create an inner microtubule scaffold, likely providing mechanical support for axonemal microtubules to withstand shear forces from the extracellular environment. Studies using native extracted microtubule networks showed that kinesin-1 moves preferentially on acetylated microtubules (Balabanian et al. 2017, Tas et al. 2017). However, consistent with its luminal

location, acetylation does not directly affect kinesin-1 binding or motility in vitro (Balabanian et al. 2017). One resolution to this discrepancy is that bundled microtubules in cells are enriched in acetylation, and kinesin-1 motility is enhanced on microtubule bundles (Balabanian et al. 2017). This preference for bundled acetylated microtubules contributes to the axonal targeting of this motor in neurons (Tas et al. 2017).

Less is known about methylation readers. Reduction of SETD2 levels disrupts the interaction between tubulin and polybromo 1 (PBRM1), a histone code reader involved in chromosome stability and a strong tumor suppressor. Thus, PBRM1 may be a reader and integrator of both the tubulin and histone codes (Karki et al. 2021) (**Figure 3**).

3.2.4. Cellular functions and disease. K40 acetylation is especially abundant in axonemes and increases as cilia mature (LeDizet & Piperno 1991, Mahecic et al. 2020), but, surprisingly, neither overexpression of nonacetylatable α-tubulin K40R in ciliates (Kozminski et al. 1993, Gaertig et al. 1995) nor TAT knockout in mice elicits major phenotypes, though mice display brain deformation in the dentate gyrus, suggesting that acetylation is important for neurogenesis and cell migration (Kim et al. 2013) (**Figure 3**). Still, control of tubulin acetylation is essential for several cellular functions and organismal fitness. Genetic knockout and drug studies in mammalian cell culture showed that HDAC6 activity is required to mediate focal adhesion dynamics for rapid cell migration (Tran et al. 2007) and that a decrease in TAT activity impairs motility of primary rat astrocytes by reducing Rab6 vesicle fusion at focal adhesions and inhibiting their turnover (Bance et al. 2019) (**Figure 3**). mec-17/TAT knockout in *C. elegans* (Shida et al. 2010) and mice (Morley et al. 2016) showed that K40 acetylation is necessary for mechanosensation. Moreover, mec-17 knockout in *C. elegans* disrupts axon transport and causes neurodegeneration, demonstrating that acetylation is essential to maintain axonal integrity (Neumann & Hilliard 2014).

Homozygous *Setd2* mutations that selectively abolish tubulin methylation are lethal in mice, and heterozygous mice exhibit autism-related behaviors, with primary neuronal cultures displaying defects in axon length and arborization (Koenning et al. 2021) (**Figure 3**). SETD2 loss also causes genomic instability associated with cancer pathogenesis (Gerlinger et al. 2012, Carvalho et al. 2014); however, it remains to be established whether these phenotypes are due to the effects on tubulin, as opposed to histones.

3.3. Phosphorylation

Phosphorylation involves the addition of a phosphate group. It has been detected at multiple tyrosine and serine residues in α- and β-tubulin.

3.3.1. Writers/erasers. Tubulin phosphorylation was identified in 1972, and with few exceptions, its functional significance remains unclear (Eipper 1972), though several tubulin kinases are known. Nonreceptor tyrosine kinase (Src) phosphorylates tubulin in fractionated nerve growth cone membranes (Matten et al. 1990), Syk kinase phosphorylates α-tubulin Y432 located on the C-terminal tail in vitro and in B lymphocytes, and protein kinase C (PKC) phosphorylates α-tubulin S165 (**Figure 1**). The best-characterized tubulin phosphosite is β-tubulin S172, phosphorylated by cyclin-dependent kinase 1 (CDK1) (Fourest-Lieuvin et al. 2006, Caudron et al. 2010) and the Down syndrome–associated kinase dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) (Ori-McKenney et al. 2016). This residue is conserved and phosphorylation at this site is enriched in soluble tubulin, suggesting a kinase preference for the soluble tubulin dimer over the microtubule.

3.3.2. Direct effects on microtubule properties. The effects of most phosphosites on microtubule properties have yet to be determined. β-tubulin S172 phosphorylation by CDK1

or DYRK1A reduces incorporation into microtubules in vitro (Fourest-Lieuvin et al. 2006, Ori-McKenney et al. 2016). This is likely due to the phosphate's proximity to the β-tubulin GTP-binding site, which controls microtubule dynamics. Conversely, PKC phosphorylation of α-tubulin S165 promotes microtubule polymerization in vitro (De et al. 2014).

3.3.3. Readers. Identification of tubulin phosphorylation readers remains an underexplored field. One study used an α-tubulin S165 phosphomimetic to assess how constitutive phosphorylation affects the small GTPases CDC42, Rac1, and RhoA and found higher Rac1 activation and enhanced cell motility in human cancer cells (De et al. 2014) (**Figure 3**).

3.3.4. Cellular functions and disease. Both of the phosphosites known to modulate intrinsic parameters of microtubules have strong associations with disease. β-tubulin S172 mutations disrupt cell migration and axon outgrowth, causing structural brain abnormalities (Jaglin et al. 2009) (**Figure 3**). DYRK1A (MNB in flies) was identified as the β-tubulin S172 kinase in a *Drosophila* screen, where its loss dramatically disrupted dendritic arborization (Ori-McKenney et al. 2016). Subsequent studies showed that DYRK1A haploinsufficiency causes similar phenotypes in the mouse cortex (Arranz et al. 2019) and that DYRK1A regulates axonal transport in human neurons (Fernandez Bessone et al. 2022) (**Figure 3**).

3.4. Glutamylation

Glutamylation involves the addition of glutamate residues, either singly or sequentially in chains of variable lengths, to internal glutamate residues in α- and β-tubulin.

3.4.1. Writers/Erasers. Glutamylation is found on multiple glutamates in the intrinsically disordered tubulin C-terminal tails (Audebert et al. 1993, 1994; Redeker et al. 1994; Bobinnec et al. 1998). The different positions on the tubulin tail, as well as the range of polyglutamate chain lengths, allow complex, graded, and combinatorial action for this modification (Roll-Mecak 2020). As many as 11 and 7 glutamates have been detected on brain α-tubulin and β-tubulin, respectively (Redeker 2010), while ciliary microtubules have up to 21 glutamates (Schneider et al. 1998). Thus, glutamylation changes both the reach of the tubulin tail (in terms of overall length, glutamate chains can be longer than the tubulin tail from which they originate) and its electrostatic charge, suggesting vast potential to alter microtubule properties.

Glutamylation is catalyzed by TTL-like (TTLL) enzymes, which are homologous to TTL in their core domains (Janke et al. 2005, Van Dijk et al. 2007) and possess accessory domains that target their substrates (Garnham et al. 2015, Sun et al. 2016) (**Figure 1**). Glutamylases prefer either α- or β-tubulin. TTLL1, -5, -6, -9, -11, and -13 preferentially glutamylate α-tubulin (Van Dijk et al. 2007, Mahalingan et al. 2020, Bodakuntla et al. 2021), and TTLL4 and -7 glutamylate β-tubulin (Ikegami et al. 2006; Van Dijk et al. 2007; Garnham et al. 2015, 2017; Mahalingan et al. 2020). The structure of TTLL7 with the microtubule led to the identification of a unique microtubule-binding domain shared among several autonomous TTLL glutamylases, except for TTLL1, which functions as part of a multisubunit complex. This domain makes polymer-specific contacts in TTLL7 that dictate the preference for the β-tail (Garnham et al. 2015). Structural studies of α-tubulin-specific TTLLs are needed to establish principles for tubulin protomer recognition.

Similar to the ubiquitin code, TTLL glutamylases segregate into initiases and elongases. Initiases start the glutamate chain by adding one branch-point glutamate to an internal position in the tubulin tail, while elongases extend the chain. In mammals, TTLL1, -4, and -5 are initiases (Janke et al. 2005, Ikegami et al. 2006, Van Dijk et al. 2007, Mukai et al. 2009, Mahalingan et al. 2020); TTLL6, -9, -11, and -13 are elongases (Janke et al. 2005, Ikegami et al. 2006, Van Dijk

et al. 2007, Mukai et al. 2009, Mahalingan et al. 2020); and TTLL7 is an initiase/elongase (Janke et al. 2005, Ikegami et al. 2006, Van Dijk et al. 2007, Mukai et al. 2009, Garnham et al. 2017, Mahalingan et al. 2020). NMR spectroscopy demonstrated that TTLL6 elongates α-linked glutamate chains (Mahalingan et al. 2020), and X-ray crystallography coupled with the use of initiase- and elongase-specific transition-state analog inhibitors and protein engineering identified the active-site signatures that determine initiase or α-linked elongase activity for both TTLL glutamylases and glycylases (Mahalingan et al. 2020). These active-site signatures can be used to predict the biochemical activity of unannotated TTLL genes, which is difficult to do on the basis of homology alone because of the high level of conservation among TTLLs.

Deglutamylation is catalyzed by CCPs (Rogowski et al. 2010) (**Figure 1**). The carboxypeptidases CCP1, -4, and -6 catalyze the removal of long glutamate chains, and CCP5 catalyzes the removal of branch-point glutamates. Thus, the combinatorial action of TTLL glutamylases and CCP deglutamylases creates dynamic, complex glutamate patterns on microtubules. TTLLs and CCPs also modify nontubulin substrates, including nucleosome assembly proteins (Regnard et al. 2000) and myosin light-chain kinase 1 (Rogowski et al. 2010), respectively. TTLL5 glutamylates the retinitis pigmentosa GTPase regulator (RPGR), whose function in photoreceptors requires glutamylation, as TTLL5 loss phenocopies RPGR loss and causes retinal degeneration in mice (Sun et al. 2016) and retinal dystrophy in humans (Smirnov et al. 2021).

There may be significant crosstalk between glutamylation and detyrosination. Glutamylated and detyrosinated regions of microtubules frequently overlap in cells, and detyrosination increases upon TTLL5/TTLL6 overexpression (Ebberink et al. 2022). Consistent with feedback between these two modifications, TTLL6 has significantly higher activity on detyrosinated microtubules (Mahalingan et al. 2020), and vasohibin/SVBP detyrosination activity increases with polyglutamylation in vitro (Ebberink et al. 2022).

3.4.2. Direct effects on microtubule properties. Microtubule stability and glutamylation correlate strongly in vivo, such as on axonemal microtubules or axonal microtubule bundles (Guichard et al. 2023). The direct effects of glutamylation on microtubule dynamics remained the subject of speculation for a long time due to difficulties in generating tubulin with controlled glutamylation levels for in vitro microtubule dynamics assays. Surprisingly, recent in vitro reconstitution studies demonstrate that glutamylation does not stabilize microtubules, but it inhibits microtubule growth and increases catastrophe (Chen & Roll-Mecak 2023). Thus, the correlation of glutamylation with microtubule stability in cells is indirect, through the accumulation of other modifications and/or recruitment of effectors. In vitro biophysical studies revealed that glutamylation increases the stiffness of taxol-stabilized microtubules by altering interactions between the α-tubulin C-terminal tail and the tubulin body (Wall et al. 2020). Thus, alterations to intrinsically disordered tubulin tails can affect both microtubule dynamics and mechanical properties.

3.4.3. Readers. The length and patterns of glutamate chains on tubulin tails can have complex outcomes on effectors. Kinesin-1 (KIF5B) motility is selectively enhanced on microtubules with long glutamate chains but not short ones, whereas kinesin-2 (KIF17) motility is enhanced on microtubules regardless of glutamate chain length (Sirajuddin et al. 2014). The addition of long glutamate chains to microtubules stimulates the microtubule-severing enzyme spastin (Lacroix et al. 2010, Valenstein & Roll-Mecak 2016) (**Figures 1*d*** and **3**). Glutamylation on β-tubulin quantitatively regulates spastin-mediated microtubule severing in a biphasic fashion, as a function of the number of glutamates. Given the stereotyped distribution of glutamylation in cells and throughout development, this substrate-mediated control provides a precise mechanism for spatial and temporal regulation of microtubule abundance and stability (Valenstein & Roll-Mecak 2016). Glutamylation also directly regulates katanin microtubule severing (**Figure 3**).

Glutamylation on the α-tail is steeply stimulatory, while modification on the β-tail controls severing in a biphasic manner, as observed for spastin (Szczesna et al. 2022). Structural modeling demonstrated that the katanin and spastin central pore that binds the glutamylated β-tail controls this biphasic response to glutamylation (Szczesna et al. 2022). The stimulatory effects of glutamylation can be counteracted by glycylation, which inhibits katanin (Szczesna et al. 2022), demonstrating that tubulin modifications can act combinatorially to elicit opposing functional outcomes. Studies in knockout mice also support the differential effects of glutamylation on the α- versus β-tubulin tails for motors that carry axonal cargo. Mitochondria in the axons of primary neurons from TTLL1 knockout mice have enhanced motility (Bodakuntla et al. 2021), and taxol-stabilized microtubules purified from TTLL7 knockout mice brains show enhanced kinesin-1 (KIF5B) binding interaction and run time (Genova et al. 2023). We note that in a purified system with yeast microtubules that had only glutamylation and no other modifications, kinesin-1 (KIF5B) showed an increase in processivity on microtubules with long glutamate chains (10 glutamates) on their β-tubulin tails and on microtubules glutamylated on α-tubulin (Sirajuddin et al. 2014) (**Figure 3**).

More generally, there is strong cellular evidence that hyperglutamylation reduces motor/cargo transport motility. CCP1 knockout primary neurons show reduced motility of various cargos (Bodakuntla et al. 2020), and increasing neuronal activity inhibits dendritic transport of the postsynaptic protein gephyrin in a glutamylase-dependent manner (Maas et al. 2009) (**Figure 3**). The activity–glutamylation–transport axis may be extremely important for neuronal homeostasis, and future studies that probe synaptic activity on shorter timescales might be fruitful since posttranslational modifications confer rapid changes in response to external cues. Interestingly, studies in *C. elegans* sensory cilia showed that (*a*) environmental stimuli, including starvation, osmolality, and temperature changes, trigger TTLL4 activation through phosphorylation by p38 MAPK and (*b*) the increase in glutamylation increases the velocity of intraflagellar transport particles (Kimura et al. 2018). It is possible that MAPK-mediated regulation is operational in other systems.

Finally, in vitro and cellular experiments show that readers involved in organelle positioning are controlled by graded glutamylation patterns. The endoplasmic reticulum (ER) membrane protein kinectin (KTN1) preferentially interacts with polyglutamylated rather than monoglutamylated microtubules, whereas the ER membrane protein p180 preferentially interacts with glutamylated microtubules more broadly. Through this graded glutamylation response, KTN1 and p180 control the distribution of the ER and other organelles, such as mitochondria and lysosomes, revealing another important cellular function of the tubulin code (Zheng et al. 2022) (**Figure 3**).

Most structural MAPs require tubulin C-terminal tails for microtubule binding, suggesting that glutamylation and MAPs cooperate to control motor/cargo motility (Roll-Mecak 2020). Glutamylation increases tau, MAP2, MAP1B, and MAP1A binding to microtubules, according to early blot overlay experiments (Boucher et al. 1994, Larcher et al. 1996, Bonnet et al. 2001) (**Figure 3**) and recent in vitro microtubule assays using tubulin purified from mouse brain lacking TTLL1, TTLL7, or CCP1 carboxypeptidase (Genova et al. 2023). The responses of many MAPs and motors to glutamylation and other modifications have yet to be determined and demand further study.

3.4.4. Cellular functions and disease. Ciliated protists, displaying abundant glutamylation on their many axonemes, provided early insights into glutamylation function. Combinatorial glutamylase knockouts in *T. thermophila* showed that glutamylation is essential for centriolar microtubule organization and morphology (Wloga et al. 2008). *Tetrahymena* research also revealed crosstalk between α- and β-tubulin tail modifications, whereby mutation of modification sites on one affected modification of the other (Redeker et al. 2005), the first such molecular analysis. Studies in *Chlamydomonas* and *Tetrahymena* also revealed that higher glutamylation levels suppress microtubule sliding by inner-arm dyneins, possibly by increasing the strength of the interaction with the microtubule (Kubo et al. 2010, Suryavanshi et al. 2010) (**Figure 3**).

Research in *C. elegans* showed that both glutamylase TTLL11 and deglutamylase CCPP1 are required for ciliary extracellular vesicle release (O'Hagan et al. 2017), and studies using zebrafish morpholinos revealed that TTLL glutamylases are important in cilia maintenance and motility (Pathak et al. 2011, 2014). TTLL1 knockout also disrupts axonemal structure, beating asymmetry, and fluid flow of tracheal cilia (Ikegami et al. 2010), and TTLL6 reduction impairs ciliary beating frequency in mouse ependymal cells (Bosch Grau et al. 2013). Interestingly, CCPP1 loss in *C. elegans* causes B-tubule instability in sensory cilia (O'Hagan et al. 2011), suggesting that the restricted localization of glutamylation is important for axonemal integrity (**Figure 3**). Consistent with model organisms, defective tubulin glutamylation causes ciliopathies in humans, such as Joubert syndrome, where *CEP41* mutations disrupt TTLL6-mediated axonemal glutamylation (Lee et al. 2012).

In proliferating cells, polyglutamylation accumulates on the mitotic spindle (Bobinnec et al. 1998), and TTLL11-mediated polyglutamylation is essential for chromosome segregation fidelity (Zadra et al. 2022). An intriguing new study showed that stiffer tumor microenvironments promote microtubule glutamylation and stabilization, followed by cell invasion (Torrino et al. 2021) (**Figure 3**). The authors of this study linked the increased glutamylation to an increase in the intracellular glutamate pool elicited by changes in glutamine metabolism. This finding indicates that metabolic rewiring, a hallmark of cancer cells, can alter microtubule dynamics by changing the balance of tubulin modifications.

Glutamylation is the most abundant tubulin modification in brain tissue, increasing steadily during development (Audebert et al. 1994). Neurons utilize the severing enzyme katanin, which is regulated by glutamylation (Szczesna et al. 2022), to amplify microtubule mass for axonal outgrowth as well as for cytoarchitecture maintenance and plasticity throughout the organisms' lifetime (**Figure 3**). Consistent with this evidence, katanin knockdown–related motor axon pathfinding errors are rescued by TTLL6 overexpression in zebrafish (Ten Martin et al. 2022). Other identified glutamylation readers function in important neuronal pathways, such as organelle positioning, cargo transport, and synaptic activity. Consistent with this functional significance, *CCP1* mutations cause early-onset neurodegeneration (Shashi et al. 2018, Karakaya et al. 2019, Sheffer et al. 2019) (**Figure 3**). Recently, tau-related neurodegeneration was also implicated. Mutation of every glutamylation site in the *TUBA4A* C-terminal tail rescued tau hyperphosphorylation, tau oligomerization, and elevated microglia activation in brain tissue in an hTau tauopathy mouse (Hausrat et al. 2022). All these studies support the importance of exquisitely controlled microtubule glutamylation for long-term neuronal health.

3.5. Glycylation

Glycylation involves the addition of glycine residues, either singly or sequentially in chains of variable lengths, to internal glutamate residues in α- and β-tubulin.

3.5.1. Writers/erasers. Tubulin glycylation was originally identified in *Paramecium*, which has thousands of cilia with high glycylation levels (Redeker et al. 1994). Ciliary microtubules can have chains up to 40 glycines long (Wall et al. 2016). Like glutamylation, glycylation is catalyzed by TTLL enzymes functioning as either initiases or elongases (**Figure 1**). In mammals, TTLL3 and -8 initiate glycine chains, forming an isopeptide bond between the internal glutamate and the incoming glycine, whereas TTLL10 elongates these chains through the formation of canonical peptide bonds (Ikegami & Setou 2009, Rogowski et al. 2009, Garnham et al. 2017) (**Figure 1**). The two TTLL3 homologs in *Drosophila* are capable of initiating and elongating glycine chains (Rogowski et al. 2009), which is surprising because the γ-carboxyl of glutamate (initiation) and

the α-carboxyl of glycine (elongation) present markedly different substrates to the enzyme. It is unclear whether other organisms have glycylases with similar flexibility in substrate recognition.

Although two metallocarboxypeptidase enzymes capable of shortening polyglycine chains were discovered in *Giardia duodenalis* (Lalle et al. 2011), no homologs have been found in animals. *Paramecium* cell extract experiments suggest the presence of a deglycylating enzyme (Bre et al. 1998); however, no specific tubulin-deglycylating enzyme has been confirmed in any organism.

3.5.2. Direct effects on microtubule properties. Glycylation effects on intrinsic microtubule properties are largely unknown. Surprisingly, in vitro biophysical studies showed that glycylation decreases microtubule stiffness (Wall et al. 2020). The effect of glycylation on microtubule dynamics is unknown.

3.5.3. Readers. All in vivo analyses of glycylation must consider the anticorrelation of glutamylation and glycylation, primarily due to shared modification sites. For example, glutamylase TTLL7 and glycylase TTLL3 target overlapping residues on β-tubulin tails (Garnham et al. 2017), and manipulating one modification typically upregulates the other (Rogowski et al. 2009), making in vitro reconstitution critical for mechanistic insight. The direct effects of glycylation on microtubule effectors is mostly unexplored, largely due to the inability to generate in vitro microtubules with defined glycylation levels. The first in vitro reconstitution of microtubule mono- and polyglycylation was recently reported, establishing that glycylation inhibits katanin–microtubule binding and severing even on previously glutamylated microtubules (Szczesna et al. 2022); this observation suggests a mechanism for increasing the stability of long-lived microtubules, such as those found in axonemes. Because many motors and MAPs recognize microtubules through electrostatic interactions with the negatively charged tubulin tails, it is likely that glycylation acts as a more general inhibitor for the recruitment of effectors. Cryo-electron tomography of axonemes from the sperm of mice lacking glycylation showed conformational changes in axonemal dynein arms, consistent with abnormal beating patterns of these sperm flagella (Bodakuntla et al. 2021). It is unclear whether these effects are due to glycylation loss or indirect changes in glutamylation. Further study is required to better understand the molecular and cellular mechanisms of glycylation.

3.5.4. Cellular functions and disease. The most heavily glycylated microtubules are found in the axonemes of cilia and flagella (Redeker et al. 1994, Bre et al. 1996, Xia et al. 2000, Bosch Grau et al. 2013, Rocha et al. 2014), although glycylated microtubules have also been observed in the cytoplasm and cortex of *Paramecium* (Bre et al. 1998), in *Tetrahymena* (Thazhath et al. 2002), and in the marginal band of induced pluripotent stem cell–derived proplatelet-forming megakaryocytes (Khan et al. 2022) (**Figure 3**). Consistent with this high abundance, glycylation defects influence ciliary function in many organisms. *TTLL3* deletion in *Tetrahymena* disrupts axonemal structure, eliciting shortened cilia (Rogowski et al. 2009). Similarly, TTLL3 depletion in zebrafish leads to cilia shortening or loss in several organs, as well as defects in left–right symmetry during development (Rogowski et al. 2009) (**Figure 3**). In mice, TTLL3 and TTLL8 are required for stability and maintenance of motile ependymal cilia (Bosch Grau et al. 2013) and for robust primary cilia formation (Rocha et al. 2014). Loss of TTLL3 in mouse colon epithelium increases cell proliferation, likely due to defective cilia, and promotes tumorigenesis (Rocha et al. 2014). *TTLL10* mutations are associated with bleeding disorders in humans (Khan et al. 2022), which is particularly intriguing, since human TTLL10 was shown to be inactive (Rogowski et al. 2009). The molecular mechanisms for most of these phenotypes remain to be explored.

4. THE FUTURE OF THE TUBULIN CODE

4.1. Pattern Generation: From Single Residues to Micrometer-Scale Arrays

The generation, maintenance, and plasticity of complex isotype patterns constituting a cellular microtubule array are not yet well understood. The stereotyped modification patterns observed in cells, varying with cell type and developmental stage, strongly suggest establishment by high-fidelity developmental programs. Enzyme expression levels (**Figure 2**) and subcellular localization are basic mechanisms for controlling modification patterns, but they cannot easily explain how adjacent microtubules acquire different modifications (**Figure 3**). Moreover, many modification enzymes have diffuse cytoplasmic localization. Kinetic control, the intersection between enzyme catalytic rate and microtubule lifetime, is likely key to generating the complex modification patterns observed in cells. Such kinetic control was initially proposed for TAT due to its low catalytic rate and microtubule substrate preference (Szyk et al. 2014) and, more recently, for detyrosination (Chen et al. 2021), whereby kinetic control can elicit accumulation on longer-lived microtubules, even though the enzyme action is stochastic, and can generate distinct modification signatures on even closely juxtaposed microtubules. MAPs also affect microtubule lifetime, can block modification site accessibility, and serve as scaffolds for enzyme recruitment (Bompard et al. 2018), adding another layer of control over modification patterns. Additionally, competition for modification sites and crosstalk between modifications can further refine patterns, whereby an initial modification elicits the accumulation (or reduction) of additional modifications (Rogowski et al. 2009, Ebberink et al. 2022), modulating microtubule fate. Lastly, metabolic signaling, cellular stress, or synaptic signaling can regulate local modification enzyme activity, resulting in complex spatial patterns. The molecular mechanisms behind this complex pattern generation remain largely unexplored.

4.2. Pattern Visualization: Recent Advances and Remaining Challenges

Precise characterization of modification patterns and isoform distribution in microtubule arrays has been challenging because of the need for high-resolution techniques to distinguish individual microtubules in the dense microtubule arrays often enriched in modifications. Here, recent developments in expansion microscopy offer new insights. For example, expansion microscopy coupled with stimulated emission depletion revealed a radial distribution of modifications in the dendrite, with acetylated microtubules enriched in the central core and tyrosinated microtubules enriched near the plasma membrane (Katrukha et al. 2021) (**Figure 3**). Thus, what was thought to be merely enrichment in these two modifications is, in fact, a precisely defined nanoscale pattern carrying functional implications, with the acetylated core region possibly acting as a dedicated highway for efficient transport and the tyrosinated outer region acting as a responsive scaffold, facilitating branch dynamics and synaptic plasticity. Expansion microscopy also revealed the finer modification patterns on centrioles and cilia (Damstra et al. 2022, Louvel et al. 2022), for example, by revealing the distinctive glutamylation distribution along the human centriole, whereby glutamylation is restricted to the A tubule at the proximal end and the C tubule at the distal end (Mahecic et al. 2020), with functional implications for the MAPs and motors in these structures.

Despite these recent advances, modification pattern insights continue to be hampered by limited availability of high-affinity, specific antibodies for many isoforms and modifications. Moreover, the graded response to polyglutamylation and polyglycylation of microtubule effectors (Sirajuddin et al. 2014, Valenstein & Roll-Mecak 2016, Szczesna et al. 2022, Zheng et al. 2022) underscores the importance of quantitatively determining the glutamate or glycine chain length with reporters. Antibodies are unlikely to achieve this resolution, but modification enzymes engineered to accept modified substrates suitable for click chemistry have this potential. The

structural knowledge gained on modification enzyme active sites (Prota et al. 2013, Szyk et al. 2014, Li et al. 2019, Wang et al. 2019, Mahalingan et al. 2020) will be instrumental for such efforts. Microtubules and their modifications are dynamic, and live reporters for modifications are still largely missing, excepting tyrosination (Kesarwani et al. 2020). A new generation of chemists may apply their tools to this interesting but challenging problem.

4.3. Cellular Readout of Modification Patterns

Cellular microtubule functions integrate every molecular component affecting the microtubule cytoskeleton, from chaperones to MAPs, motors, and motor adaptor proteins. This complexity means that much is left to explore regarding the effects the tubulin code exerts on molecular effectors, cellular pathways, and human physiology (**Figure 3**). Phenotypes associated with mutations, reduction, or overexpression of individual isoforms or enzymes in vivo must be carefully interpreted because many modification enzymes modify nontubulin substrates and, especially with isoforms and TTLLs, homolog compensation may confound results. Thus, in vitro reconstitution experiments will continue to be invaluable to establish causality. The gradual move toward the use of isotypically pure recombinant tubulin (Minoura et al. 2013, Vemu et al. 2016, Ti et al. 2018, Diao et al. 2021) and tubulin with well-defined modifications, through enzymatic modification of unmodified tubulin (Vemu et al. 2014, Valenstein & Roll-Mecak 2016, Szczesna et al. 2022, Zheng et al. 2022), genetic engineering (Chen et al. 2021), or semisynthetic approaches (Ebberink et al. 2022), now allows in vitro reconstitution to test mechanistic tubulin code hypotheses. Moreover, more sophisticated cellular models, including diverse cell types and organoids derived from stem cells, enable us to capture nuanced two- and three-dimensional phenotypes. Gene editing, which allows modification of only targeted isoform residues or substitution of isoforms at endogenous expression levels, will be invaluable for dissecting the contribution of individual isoforms and modifications in vivo.

Taken together, these technological advances enable exciting leaps in understanding the tubulin code, both the mechanisms that give rise to it and the physiological outcomes that arise from it. With the use of in vitro reconstitution assays combined with in vivo gene editing approaches and advanced imaging, the coming years appear ripe to shed light on how cells functionalize their microtubule cytoskeletons to support the complex biological processes they control, with direct implications for understanding human disease.

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LITERATURE CITED

Adamopoulos A, Landskron L, Heidebrecht T, Tsakou F, Bleijerveld OB, et al. 2019. Crystal structure of the tubulin tyrosine carboxypeptidase complex VASH1-SVBP. *Nat. Struct. Mol. Biol.* 26:567–70

Aillaud C, Bosc C, Peris L, Bosson A, Heemeryck P, et al. 2017. Vasohibins/SVBP are tubulin carboxypeptidases (TCPs) that regulate neuron differentiation. *Science* 358:1448–53

Aillaud C, Bosc C, Saoudi Y, Denarier E, Peris L, et al. 2016. Evidence for new C-terminally truncated variants of α- and β-tubulins. *Mol. Biol. Cell* 27:640–53

Akella JS, Wloga D, Kim J, Starostina NG, Lyons-Abbott S, et al. 2010. MEC-17 is an α-tubulin acetyltransferase. *Nature* 467:218–22

Akera T, Chmatal L, Trimm E, Yang K, Aonbangkhen C, et al. 2017. Spindle asymmetry drives non-Mendelian chromosome segregation. *Science* 358:668–72

Arranz J, Balducci E, Arato K, Sánchez-Elexpuru G, Najas S, et al. 2019. Impaired development of neocortical circuits contributes to the neurological alterations in *DYRK1A* haploinsufficiency syndrome. *Neurobiol. Dis.* 127:210–22

Audebert S, Desbruyeres E, Gruszczynski C, Koulakoff A, Gros F, et al. 1993. Reversible polyglutamylation of α- and β-tubulin and microtubule dynamics in mouse brain neurons. *Mol. Biol. Cell* 4:615–26

Audebert S, Koulakoff A, Berwald-Netter Y, Gros F, Denoulet P, Edde B. 1994. Developmental regulation of polyglutamylated α- and β-tubulin in mouse brain neurons. *J. Cell Sci.* 107(Part 8):2313–22

Bachanova V, Frankel AE, Cao Q, Lewis D, Grzywacz B, et al. 2015. Phase I study of a bispecific ligand-directed toxin targeting CD22 and CD19 (DT2219) for refractory B-cell malignancies. *Clin. Cancer Res.* 21:1267–72

Balabanian L, Berger CL, Hendricks AG. 2017. Acetylated microtubules are preferentially bundled leading to enhanced kinesin-1 motility. *Biophys. J.* 113:1551–60

Bance B, Seetharaman S, Leduc C, Boeda B, Etienne-Manneville S. 2019. Microtubule acetylation but not detyrosination promotes focal adhesion dynamics and astrocyte migration. *J. Cell Sci.* 132:jcs225805

Banerjee A, Roach MC, Trcka P, Ludueña RF. 1990. Increased microtubule assembly in bovine brain tubulin lacking the type III isotype of β-tubulin. *J. Biol. Chem.* 265:1794–99

Barbosa DJ, Duro J, Prevo B, Cheerambathur DK, Carvalho AX, Gassmann R. 2017. Dynactin binding to tyrosinated microtubules promotes centrosome centration in *C. elegans* by enhancing dynein-mediated organelle transport. *PLOS Genet.* 13:e1006941

Barisic M, Silva E, Sousa R, Tripathy SK, Magiera MM, et al. 2015. Mitosis: Microtubule detyrosination guides chromosomes during mitosis. *Science* 348:799–803

Bartolomeo MD, Raimondi A, Cecchi F, Catenacci DVT, Schwartz S, et al. 2020. Association of high TUBB3 with resistance to adjuvant docetaxel-based chemotherapy in gastric cancer: translational study of ITACA-S. *Tumori J.* 107:150–59

Bhattacharya R, Cabral F. 2004. A ubiquitous β-tubulin disrupts microtubule assembly and inhibits cell proliferation. *Mol. Biol. Cell* 15:3123–31

Bieling P, Kandels-Lewis S, Telley IA, Van Dijk J, Janke C, Surrey T. 2008. CLIP-170 tracks growing microtubule ends by dynamically recognizing composite EB1/tubulin-binding sites. *J. Cell Biol.* 183:1223–33

Blair JD, Hockemeyer D, Doudna JA, Bateup HS, Floor SN. 2017. Widespread translational remodeling during human neuronal differentiation. *Cell Rep.* 21:2005–16

Bobinnec Y, Moudjou M, Fouquet JP, Desbruyeres E, Edde B, Bornens M. 1998. Glutamylation of centriole and cytoplasmic tubulin in proliferating non-neuronal cells. *Cell Motil. Cytoskelet.* 39:223–32

Bodakuntla S, Schnitzler A, Villablanca C, González-Billault C, Bieche I, et al. 2020. Tubulin polyglutamylation is a general traffic-control mechanism in hippocampal neurons. *J. Cell Sci.* 133:jcs241802

Bodakuntla S, Yuan X, Genova M, Gadadhar S, Leboucher S, et al. 2021. Distinct roles of α- and β-tubulin polyglutamylation in controlling axonal transport and in neurodegeneration. *EMBO J.* 40:e108498

Bompard G, Van Dijk J, Cau J, Lannay Y, Marcellin G, et al. 2018. CSAP acts as a regulator of TTLL-mediated microtubule glutamylation. *Cell Rep.* 25:2866–77.e5

Bonnet C, Boucher D, Lazereg S, Pedrotti B, Islam K, et al. 2001. Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by tubulin polyglutamylation. *J. Biol. Chem.* 276:12839–48

Bosch Grau M, Gonzalez Curto G, Rocha C, Magiera MM, Marques Sousa P, et al. 2013. Tubulin glycylases and glutamylases have distinct functions in stabilization and motility of ependymal cilia. *J. Cell Biol.* 202:441–51

Boucher D, Larcher JC, Gros F, Denoulet P. 1994. Polyglutamylation of tubulin as a progressive regulator of in vitro interactions between the microtubule-associated protein Tau and tubulin. *Biochemistry* 33:12471–77

Bre MH, Redeker V, Quibell M, Darmanaden-Delorme J, Bressac C, et al. 1996. Axonemal tubulin polyglycylation probed with two monoclonal antibodies: widespread evolutionary distribution, appearance during spermatozoan maturation and possible function in motility. *J. Cell Sci.* 109:727–38

Bre MH, Redeker V, Vinh J, Rossier J, Levilliers N. 1998. Tubulin polyglycylation: differential posttranslational modification of dynamic cytoplasmic and stable axonemal microtubules in *Paramecium*. *Mol. Biol. Cell* 9:2655–65

Breuss M, Heng JI-T, Poirier K, Tian G, Jaglin XH, et al. 2012. Mutations in the β-tubulin gene *TUBB5* cause microcephaly with structural brain abnormalities. *Cell Rep.* 2:1554–62

Cai D, McEwen DP, Martens JR, Meyhofer E, Verhey KJ. 2009. Single molecule imaging reveals differences in microtubule track selection between kinesin motors. *PLOS Biol.* 7:e1000216

Cambray-Deakin MA, Burgoyne RD. 1990. The non-tyrosinated Mα4 α-tubulin gene product is post-translationally tyrosinated in adult rat cerebellum. *Mol. Brain Res.* 8:77–81

Carvalho S, Vitor AC, Sridhara SC, Martins FB, Raposo AC, et al. 2014. SETD2 is required for DNA double-strand break repair and activation of the p53-mediated checkpoint. *eLife* 3:e02482

Caudron F, Denarier E, Thibout-Quintana JC, Brocard J, Andrieux A, Fourest-Lieuvin A. 2010. Mutation of Ser172 in yeast β tubulin induces defects in microtubule dynamics and cell division. *PLOS ONE* 5:e13553

Chang W, Webster DR, Salam AA, Gruber D, Prasad A, et al. 2002. Alteration of the C-terminal amino acid of tubulin specifically inhibits myogenic differentiation. *J. Biol. Chem.* 277:30690–98

Chen CY, Caporizzo MA, Bedi K, Vite A, Bogush AI, et al. 2018. Suppression of detyrosinated microtubules improves cardiomyocyte function in human heart failure. *Nat. Med.* 24:1225–33

Chen J, Kholina E, Szyk A, Fedorov VA, Kovalenko I, et al. 2021. α-tubulin tail modifications regulate microtubule stability through selective effector recruitment, not changes in intrinsic polymer dynamics. *Dev. Cell* 56:2016–2028.e4

Chen J, Roll-Mecak A. 2023. Glutamylation is a negative regulator of microtubule growth. *Mol. Biol. Cell* 34:ar70

Cho Y, Cavalli V. 2012. HDAC5 is a novel injury-regulated tubulin deacetylase controlling axon regeneration. *EMBO J.* 31:3063–78

Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, et al. 2009. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 325:834–40

Christoph DC, Kasper S, Gauler TC, Loesch C, Engelhard M, et al. 2012. β_V-tubulin expression is associated with outcome following taxane-based chemotherapy in non–small cell lung cancer. *Br. J. Cancer* 107:823–30

Cleveland DW, Lopata MA, MacDonald RJ, Cowan NJ, Rutter WJ, Kirschner MW. 1980. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. *Cell* 20:95–105

Cleveland DW, Lopata MA, Sherline P, Kirschner MW. 1981. Unpolymerized tubulin modulates the level of tubulin mRNAs. *Cell* 25:537–46

Cushion TD, Paciorkowski AR, Pilz DT, Mullins JGL, Seltzer LE, et al. 2014. De novo mutations in the β-tubulin gene *TUBB2A* cause simplified gyral patterning and infantile-onset epilepsy. *Am. J. Hum. Genet.* 94:634–41

Damstra HGJ, Mohar B, Eddison M, Akhmanova A, Kapitein LC, Tillberg PW. 2022. Visualizing cellular and tissue ultrastructure using Ten-fold Robust Expansion Microscopy (TREx). *eLife* 11:e73775

Das S, Vera M, Gandin V, Singer RH, Tutucci E. 2021. Intracellular mRNA transport and localized translation. *Nat. Rev. Mol. Cell Biol.* 22:483–504

De S, Tsimounis A, Chen X, Rotenberg SA. 2014. Phosphorylation of α-tubulin by protein kinase C stimulates microtubule dynamics in human breast cells. *Cytoskeleton* 71:257–72

Diao L, Liu M-Y, Song Y-L, Zhang X, Liang X, Bao L. 2021. α1A and α1C form microtubules to display distinct properties mainly mediated by their C-terminal tails. *J. Mol. Cell Biol.* 13:mjab062

Dunn S, Morrison EE, Liverpool TB, Molina-Paris C, Cross RA, et al. 2008. Differential trafficking of Kif5c on tyrosinated and detyrosinated microtubules in live cells. *J. Cell Sci.* 121:1085–95

Ebberink E, Fernandes S, Hatzopoulos G, Agashe N, Guidotti N, et al. 2022. Tubulin engineering by semisynthesis reveals that polyglutamylation directs detyrosination. bioRxiv 2022.09.20.508649. **https://doi.org/10.1101/2022.09.20.508649**

Eipper BA. 1972. Rat brain microtubule protein: purification and determination of covalently bound phosphate and carbohydrate. *PNAS* 69:2283–87

Erck C, Peris L, Andrieux A, Meissirel C, Gruber AD, et al. 2005. A vital role of tubulin-tyrosine-ligase for neuronal organization. *PNAS* 102:7853–58

Eshun-Wilson L, Zhang R, Portran D, Nachury MV, Toso DB, et al. 2019. Effects of α-tubulin acetylation on microtubule structure and stability. *PNAS* 116:10366–71

Feng R, Yan Z, Li B, Yu M, Sang Q, et al. 2016. Mutations in *TUBB8* cause a multiplicity of phenotypes in human oocytes and early embryos. *J. Med. Genet.* 53:662–71

Fernandez Bessone I, Navarro J, Martinez E, Karmirian K, Holubiec M, et al. 2022. *DYRK1A* regulates the bidirectional axonal transport of APP in human-derived neurons. *J. Neurosci.* 42:6344–58

Fourest-Lieuvin A, Peris L, Gache V, Garcia-Saez I, Juillan-Binard C, et al. 2006. Microtubule regulation in mitosis: tubulin phosphorylation by the cyclin-dependent kinase Cdk1. *Mol. Biol. Cell* 17:1041–50

Fukushige T, Siddiqui ZK, Chou M, Culotti JG, Gogonea CB, et al. 1999. MEC-12, an α-tubulin required for touch sensitivity in *C. elegans*. *J. Cell Sci.* 112:395–403

Fulton C. 2022. The amazing evolutionary complexity of eukaryotic tubulins: lessons from naegleria and the multi-tubulin hypothesis. *Front. Cell Dev. Biol.* 10:867374

Fulton C, Simpson PA. 1976. Selective synthesis and utilization of flagellar tubulin: the multi-tubulin hypothesis. In *Cell Motility*, ed. R Goldman, T Pollard, J Rosenbaum, pp. 987–1005. Cold Spring Harbor, NY: Cold Spring Harbor Lab Press

Gaertig J, Cruz MA, Bowen J, Gu L, Pennock DG, Gorovsky MA. 1995. Acetylation of lysine 40 in α-tubulin is not essential in *Tetrahymena thermophila*. *J. Cell Biol.* 129:1301–10

Gao S, Wang S, Zhao Z, Zhang C, Liu Z, et al. 2022. TUBB4A interacts with MYH9 to protect the nucleus during cell migration and promotes prostate cancer via GSK3β/β-catenin signalling. *Nat. Commun.* 13:2792

Garnham CP, Vemu A, Wilson-Kubalek EM, Yu I, Szyk A, et al. 2015. Multivalent microtubule recognition by tubulin tyrosine ligase–like family glutamylases. *Cell* 161:1112–23

Garnham CP, Yu I, Li Y, Roll-Mecak A. 2017. Crystal structure of tubulin tyrosine ligase–like 3 reveals essential architectural elements unique to tubulin monoglycylases. *PNAS* 114:6545–50

Genova M, Grycova L, Puttrich V, Magiera MM, Lansky Z, et al. 2023. Tubulin polyglutamylation differentially regulates microtubule-interacting proteins. *EMBO J.* 42:e112101

Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, et al. 2012. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* 366:883–92

Guichard P, Laporte MH, Hamel V. 2023. The centriolar tubulin code. *Semin. Cell Dev. Biol.* 137:16–25

Gumy LF, Yeo GSH, Tung Y-CL, Zivraj KH, Willis D, et al. 2011. Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localization. *RNA* 17:85–98

Gundersen GG, Bulinski JC. 1986. Distribution of tyrosinated and nontyrosinated α-tubulin during mitosis. *J. Cell Biol.* 102:1118–26

Hausrat TJ, Janiesch PC, Breiden P, Lutz D, Hoffmeister-Ullerich S, et al. 2022. Disruption of tubulin-α4a polyglutamylation prevents aggregation of hyper-phosphorylated tau and microglia activation in mice. *Nat. Commun.* 13:4192

Hebebrand M, Hüffmeier U, Trollmann R, Hehr U, Uebe S, et al. 2019. The mutational and phenotypic spectrum of *TUBA1A*-associated tubulinopathy. *Orphanet J. Rare Dis.* 14:38

Hirst WG, Biswas A, Mahalingan KK, Reber S. 2020. Differences in intrinsic tubulin dynamic properties contribute to spindle length control in *Xenopus* species. *Curr. Biol.* 30:2184–90.e5

Hoff KJ, Neumann AJ, Moore JK. 2022. The molecular biology of tubulinopathies: understanding the impact of variants on tubulin structure and microtubule regulation. *Front. Cell. Neurosci.* 16:1023267

Horio T, Hotani H. 1986. Visualization of the dynamic instability of individual microtubules by dark-field microscopy. *Nature* 321:605–7

Hotta T, McAlear TS, Yue Y, Higaki T, Haynes SE, et al. 2022. EML2-S constitutes a new class of proteins that recognizes and regulates the dynamics of tyrosinated microtubules. *Curr. Biol.* 32:3898–910.e14

Hoyle HD, Hutchens JA, Turner FR, Raff EC. 1995. Regulation of β-tubulin function and expression in *Drosophila* spermatogenesis. *Dev. Genet.* 16:148–70

Hoyle HD, Raff EC. 1990. Two *Drosophila* β tubulin isoforms are not functionally equivalent. *J. Cell Biol.* 111:1009–26

Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, et al. 2002. HDAC6 is a microtubule-associated deacetylase. *Nature* 417:455–58

Ikegami K, Mukai M, Tsuchida J, Heier RL, Macgregor GR, Setou M. 2006. TTLL7 is a mammalian β-tubulin polyglutamylase required for growth of MAP2-positive neurites. *J. Biol. Chem.* 281:30707–16

Ikegami K, Sato S, Nakamura K, Ostrowski LE, Setou M. 2010. Tubulin polyglutamylation is essential for airway ciliary function through the regulation of beating asymmetry. *PNAS* 107:10490–95

Ikegami K, Setou M. 2009. TTLL10 can perform tubulin glycylation when co-expressed with TTLL8. *FEBS Lett.* 583:1957–63

Iqbal Z, Tawamie H, Ba W, Reis A, Al Halak B, et al. 2019. Loss of function of SVBP leads to autosomal recessive intellectual disability, microcephaly, ataxia, and hypotonia. *Genet. Med.* 21:1790–96

Jaglin XH, Poirier K, Saillour Y, Buhler E, Tian G, et al. 2009. Mutations in the β-tubulin gene *TUBB2B* result in asymmetrical polymicrogyria. *Nat. Genet.* 41:746–52

Janke C, Rogowski K, Wloga D, Regnard C, Kajava AV, et al. 2005. Tubulin polyglutamylase enzymes are members of the TTL domain protein family. *Science* 308:1758–62

Jensen-Smith HC, Ludueña RF, Hallworth R. 2003. Requirement for the β_I and β_{IV} tubulin isotypes in mammalian cilia. *Cell Motil. Cytoskelet.* 55:213–20

Jiang L, Zhu X, Yang H, Chen T, Lv K. 2020. Bioinformatics analysis discovers microtubular tubulin beta 6 class V (*TUBB6*) as a potential therapeutic target in glioblastoma. *Front. Genet.* 11:566579

Karakaya M, Paketci C, Altmueller J, Thiele H, Hoelker I, et al. 2019. Biallelic variant in *AGTPBP1* causes infantile lower motor neuron degeneration and cerebellar atrophy. *Am. J. Med. Genet. A* 179:1580–84

Karki M, Jangid RK, Anish R, Seervai RNH, Bertocchio JP, et al. 2021. A cytoskeletal function for PBRM1 reading methylated microtubules. *Sci. Adv.* 7:eabf2866

Katrukha EA, Jurriens D, Salas Pastene DM, Kapitein LC. 2021. Quantitative mapping of dense microtubule arrays in mammalian neurons. *eLife* 10:e67925

Kearns S, Mason FM, Rathmell WK, Park IY, Walker C, et al. 2021. Molecular determinants for α-tubulin methylation by SETD2. *J. Biol. Chem.* 297:100898

Kemphues KJ, Kaufman TC, Raff RA, Raff EC. 1982. The testis-specific β-tubulin subunit in *Drosophila melanogaster* has multiple functions in spermatogenesis. *Cell* 31:655–70

Kesarwani S, Lama P, Chandra A, Reddy PP, Jijumon AS, et al. 2020. Genetically encoded live-cell sensor for tyrosinated microtubules. *J. Cell Biol.* 219:e201912107

Khan AO, Slater A, Maclachlan A, Nicolson PLR, Pike JA, et al. 2022. Post-translational polymodification of β_1-tubulin regulates motor protein localisation in platelet production and function. *Haematologica* 107:243–59

Kim GW, Li L, Ghorbani M, You L, Yang XJ. 2013. Mice lacking α-tubulin acetyltransferase 1 are viable but display α-tubulin acetylation deficiency and dentate gyrus distortion. *J. Biol. Chem.* 288:20334–50

Kimura Y, Tsutsumi K, Konno A, Ikegami K, Hameed S, et al. 2018. Environmental responsiveness of tubulin glutamylation in sensory cilia is regulated by the p38 MAPK pathway. *Sci. Rep.* 8:8392

Koenning M, Wang X, Karki M, Jangid RK, Kearns S, et al. 2021. Neuronal SETD2 activity links microtubule methylation to an anxiety-like phenotype in mice. *Brain* 144:2527–40

Kormendi V, Szyk A, Piszczek G, Roll-Mecak A. 2012. Crystal structures of tubulin acetyltransferase reveal a conserved catalytic core and the plasticity of the essential N terminus. *J. Biol. Chem.* 287:41569–75

Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, et al. 2005. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol. Cell* 18:601–7

Kozminski KG, Diener DR, Rosenbaum JL. 1993. High level expression of nonacetylatable α-tubulin in *Chlamydomonas reinhardtii*. *Cell Motil. Cytoskelet.* 25:158–70

Kreis TE. 1987. Microtubules containing detyrosinated tubulin are less dynamic. *EMBO J.* 6:2597–606

Kreitzer G, Liao G, Gundersen GG. 1999. Detyrosination of tubulin regulates the interaction of intermediate filaments with microtubules in vivo via a kinesin-dependent mechanism. *Mol. Biol. Cell* 10:1105–18

Kubo T, Yanagisawa HA, Yagi T, Hirono M, Kamiya R. 2010. Tubulin polyglutamylation regulates axonemal motility by modulating activities of inner-arm dyneins. *Curr. Biol.* 20:441–45

Kunishima S, Kobayashi R, Itoh TJ, Hamaguchi M, Saito H. 2009. Mutation of the β_1-tubulin gene associated with congenital macrothrombocytopenia affecting microtubule assembly. *Blood* 113:458–61

L'Hernault SW, Rosenbaum JL. 1985. *Chlamydomonas* α-tubulin is posttranslationally modified by acetylation on the ε-amino group of a lysine. *Biochemistry* 24:473–78

Lacroix B, Van Dijk J, Gold ND, Guizetti J, Aldrian-Herrada G, et al. 2010. Tubulin polyglutamylation stimulates spastin-mediated microtubule severing. *J. Cell Biol.* 189:945–54

Lalle M, Camerini S, Cecchetti S, Blasetti Fantauzzi C, Crescenzi M, Pozio E. 2011. *Giardia duodenalis* 14-3-3 protein is polyglycylated by a tubulin tyrosine ligase–like member and deglycylated by two metallocarboxypeptidases. *J. Biol. Chem.* 286:4471–84

Landskron L, Bak J, Adamopoulos A, Kaplani K, Moraiti M, et al. 2022. Posttranslational modification of microtubules by the MATCAP detyrosinase. *Science* 376:eabn6020

Larcher JC, Boucher D, Lazereg S, Gros F, Denoulet P. 1996. Interaction of kinesin motor domains with α- and β-tubulin subunits at a tau-independent binding site. Regulation by polyglutamylation. *J. Biol. Chem.* 271:22117–24

Latham JA, Dent SY. 2007. Cross-regulation of histone modifications. *Nat. Struct. Mol. Biol.* 14:1017–24

Latremoliere A, Cheng L, DeLisle M, Wu C, Chew S, et al. 2018. Neuronal-specific TUBB3 is not required for normal neuronal function but is essential for timely axon regeneration. *Cell Rep.* 24:1865–79.e9

Leandro-García LJ, Leskelä S, Landa I, Montero-Conde C, López-Jiménez E, et al. 2010. Tumoral and tissue-specific expression of the major human β-tubulin isotypes. *Cytoskeleton* 67:214–23

LeDizet M, Piperno G. 1987. Identification of an acetylation site of *Chlamydomonas* α-tubulin. *PNAS* 84:5720–24

LeDizet M, Piperno G. 1991. Detection of acetylated α-tubulin by specific antibodies. *Methods Enzymol.* 196:264–74

Lee JE, Silhavy JL, Zaki MS, Schroth J, Bielas SL, et al. 2012. CEP41 is mutated in Joubert syndrome and is required for tubulin glutamylation at the cilium. *Nat. Genet.* 44:193–99

Li F, Hu Y, Qi S, Luo X, Yu H. 2019. Structural basis of tubulin detyrosination by vasohibins. *Nat. Struct. Mol. Biol.* 26:583–591

Li F, Li Y, Ye X, Gao H, Shi Z, et al. 2020. Cryo-EM structure of VASH1-SVBP bound to microtubules. *eLife* 9:e58157

Lin Z, Gasic I, Chandrasekaran V, Peters N, Shao S, et al. 2020. TTC5 mediates autoregulation of tubulin via mRNA degradation. *Science* 367:100–4

Lopes D, Maiato H. 2020. The tubulin code in mitosis and cancer. *Cells* 9:2356

Louvel V, Haase R, Mercey O, Laporte MH, Soldati-Favre D, et al. 2022. Nanoscopy of organelles and tissues with iterative ultrastructure expansion microscopy (iU-ExM). bioRxiv 2022.11.14.516383. **https://doi.org/10.1101/2022.11.14.516383**

Lu Q, Luduena RF. 1994. In vitro analysis of microtubule assembly of isotypically pure tubulin dimers. Intrinsic differences in the assembly properties of alpha beta II, alpha beta III, and alpha beta IV tubulin dimers in the absence of microtubule-associated proteins. *J. Biol. Chem.* 269:2041–47

Ma M, Stoyanova M, Rademacher G, Dutcher SK, Brown A, Zhang R. 2019. Structure of the decorated ciliary doublet microtubule. *Cell* 179:909–22.e12

Maas C, Belgardt D, Lee HK, Heisler FF, Lappe-Siefke C, et al. 2009. Synaptic activation modifies microtubules underlying transport of postsynaptic cargo. *PNAS* 106:8731–36

Mahalingan KK, Keenan EK, Strickland M, Li Y, Liu Y, et al. 2020. Structural basis for polyglutamate chain initiation and elongation by TTLL family enzymes. *Nat. Struct. Mol. Biol.* 27:802–13

Mahecic D, Gambarotto D, Douglass KM, Fortun D, Banterle N, et al. 2020. Homogeneous multifocal excitation for high-throughput super-resolution imaging. *Nat. Methods* 17:726–33

Maillard C, Roux CJ, Charbit-Henrion F, Steffann J, Laquerriere A, et al. 2023. Tubulin mutations in human neurodevelopmental disorders. *Semin. Cell Dev. Biol.* 137:87–95

Maruta H, Greer K, Rosenbaum JL. 1986. The acetylation of alpha-tubulin and its relationship to the assembly and disassembly of microtubules. *J. Cell Biol.* 103:571–79

Matten WT, Aubry M, West J, Maness PF. 1990. Tubulin is phosphorylated at tyrosine by pp60c-src in nerve growth cone membranes. *J. Cell Biol.* 111:1959–70

Maurin J, Morel A, Guérit D, Cau J, Urbach S, et al. 2021. The β-tubulin isotype TUBB6 controls microtubule and actin dynamics in osteoclasts. *Front. Cell Dev. Biol.* 9:778887

McKenney RJ, Huynh W, Vale RD, Sirajuddin M. 2016. Tyrosination of α-tubulin controls the initiation of processive dynein-dynactin motility. *EMBO J.* 35:1175–85

Mechaussier S, Dodd DO, Yeyati PL, McPhie F, Attard T, et al. 2022. *TUBB4B* variants specifically impact ciliary function, causing a ciliopathic spectrum. medRxiv 2022.10.19.22280748. **https://doi.org/10.1101/2022.10.19.22280748**

Minoura I, Hachikubo Y, Yamakita Y, Takazaki H, Ayukawa R, et al. 2013. Overexpression, purification, and functional analysis of recombinant human tubulin dimer. *FEBS Lett.* 587:3450–55

Mitchison T, Kirschner M. 1984. Dynamic instability of microtubule growth. *Nature* 312:237–42

Miyake Y, Keusch JJ, Wang L, Saito M, Hess D, et al. 2016. Structural insights into HDAC6 tubulin deacetylation and its selective inhibition. *Nat. Chem. Biol.* 12:748–54

Mohan N, Sorokina EM, Verdeny IV, Alvarez AS, Lakadamyali M. 2019. Detyrosinated microtubules spatially constrain lysosomes facilitating lysosome–autophagosome fusion. *J. Cell Biol.* 218:632–43

Morley SJ, Qi Y, Iovino L, Andolfi L, Guo D, et al. 2016. Acetylated tubulin is essential for touch sensation in mice. *eLife* 5:e20813

Mukai M, Ikegami K, Sugiura Y, Takeshita K, Nakagawa A, Setou M. 2009. Recombinant mammalian tubulin polyglutamylase TTLL7 performs both initiation and elongation of polyglutamylation on β-tubulin through a random sequential pathway. *Biochemistry* 48:1084–93

Neumann B, Hilliard MA. 2014. Loss of MEC-17 leads to microtubule instability and axonal degeneration. *Cell Rep.* 6:93–103

Nieuwenhuis J, Adamopoulos A, Bleijerveld OB, Mazouzi A, Stickel E, et al. 2017. Vasohibins encode tubulin detyrosinating activity. *Science* 358:1453–56

Nirschl JJ, Magiera MM, Lazarus JE, Janke C, Holzbaur EL. 2016. α-tubulin tyrosination and CLIP-170 phosphorylation regulate the initiation of dynein-driven transport in neurons. *Cell Rep.* 14:2637–52

Nogales E, Whittaker M, Milligan RA, Downing KH. 1999. High-resolution model of the microtubule. *Cell* 96:79–88

North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. 2003. The human Sir2 ortholog, SIRT2, is an NAD^+-dependent tubulin deacetylase. *Mol. Cell* 11:437–44

Nsamba ET, Bera A, Costanzo M, Boone C, Gupta ML. 2021. Tubulin isotypes optimize distinct spindle positioning mechanisms during yeast mitosis. *J. Cell Biol.* 220:e202010155

O'Hagan R, Piasecki BP, Silva M, Phirke P, Nguyen KC, et al. 2011. The tubulin deglutamylase CCPP-1 regulates the function and stability of sensory cilia in *C. elegans*. *Curr. Biol.* 21:1685–94

O'Hagan R, Silva M, Nguyen KCQ, Zhang W, Bellotti S, et al. 2017. Glutamylation regulates transport, specializes function, and sculpts the structure of cilia. *Curr. Biol.* 27:3430–41.e6

Ori-McKenney KM, McKenney RJ, Huang HH, Li T, Meltzer S, et al. 2016. Phosphorylation of β-tubulin by the Down syndrome kinase, minibrain/DYRK1A, regulates microtubule dynamics and dendrite morphogenesis. *Neuron* 90:551–63

Pagnamenta AT, Heemeryck P, Martin HC, Bosc C, Peris L, et al. 2019. Defective tubulin detyrosination causes structural brain abnormalities with cognitive deficiency in humans and mice. *Hum. Mol. Genet.* 28:3391–405

Pamula MC, Ti S-C, Kapoor TM. 2016. The structured core of human β tubulin confers isotype-specific polymerization properties. *J. Cell Biol.* 213:425–33

Panda D, Miller HP, Banerjee A, Ludueña RF, Wilson L. 1994. Microtubule dynamics in vitro are regulated by the tubulin isotype composition. *PNAS* 91:11358–62

Park IY, Powell RT, Tripathi DN, Dere R, Ho TH, et al. 2016. Dual chromatin and cytoskeletal remodeling by SETD2. *Cell* 166:950–62

Parker AL, Teo WS, Brayford S, Moorthi UK, Arumugam S, et al. 2022. β_{III}-tubulin structural domains regulate mitochondrial network architecture in an isotype-specific manner. *Cells* 11:776

Pathak N, Austin CA, Drummond IA. 2011. Tubulin tyrosine ligase–like genes *ttll3* and *ttll6* maintain zebrafish cilia structure and motility. *J. Biol. Chem.* 286:11685–95

Pathak N, Austin-Tse CA, Liu Y, Vasilyev A, Drummond IA. 2014. Cytoplasmic carboxypeptidase 5 regulates tubulin glutamylation and zebrafish cilia formation and function. *Mol. Biol. Cell* 25:1836–44

Paturle-Lafanechère L, Eddé B, Denoulet P, Van Dorsselaer A, Mazarguil H, et al. 1991. Characterization of a major brain tubulin variant which cannot be tyrosinated. *Biochemistry* 30:10523–28

Paturle-Lafanechère L, Manier M, Trigault N, Pirollet F, Mazarguil H, Job D. 1994. Accumulation of delta 2-tubulin, a major tubulin variant that cannot be tyrosinated, in neuronal tissues and in stable microtubule assemblies. *J. Cell Sci.* 107:1529–43

Peris L, Parato J, Qu X, Soleilhac JM, Lante F, et al. 2022. Tubulin tyrosination regulates synaptic function and is disrupted in Alzheimer's disease. *Brain* 145:2486–506

Peris L, Thery M, Fauré J, Saoudi Y, Lafanechère L, et al. 2006. Tubulin tyrosination is a major factor affecting the recruitment of CAP-Gly proteins at microtubule plus ends. *J. Cell Biol.* 174:839–49

Peris L, Wagenbach M, Lafanechère L, Brocard J, Moore AT, et al. 2009. Motor-dependent microtubule disassembly driven by tubulin tyrosination. *J. Cell Biol.* 185:1159–66

Poirier K, Saillour Y, Bahi-Buisson N, Jaglin XH, Fallet-Bianco C, et al. 2010. Mutations in the neuronal β-tubulin subunit *TUBB3* result in malformation of cortical development and neuronal migration defects. *Hum. Mol. Genet.* 19:4462–73

Portran D, Schaedel L, Xu Z, Thery M, Nachury MV. 2017. Tubulin acetylation protects long-lived microtubules against mechanical ageing. *Nat. Cell Biol.* 19:391–98

Prassanawar SS, Panda D. 2019. Tubulin heterogeneity regulates functions and dynamics of microtubules and plays a role in the development of drug resistance in cancer. *Biochem. J.* 476:1359–76

Preitner N, Quan J, Nowakowski DW, Hancock ML, Shi J, et al. 2014. APC is an RNA-binding protein, and its interactome provides a link to neural development and microtubule assembly. *Cell* 158:368–82

Prota AE, Magiera MM, Kuijpers M, Bargsten K, Frey D, et al. 2013. Structural basis of tubulin tyrosination by tubulin tyrosine ligase. *J. Cell Biol.* 200:259–70

Ramirez-Rios S, Choi SR, Sanyal C, Blum TB, Bosc C, et al. 2023. VASH1-SVBP and VASH2-SVBP generate different detyrosination profiles on microtubules. *J. Cell Biol.* 222:e202205096

Ramos SI, Makeyev EV, Salierno M, Kodama T, Kawakami Y, Sahara S. 2020. Tuba8 drives differentiation of cortical radial glia into apical intermediate progenitors by tuning modifications of tubulin C termini. *Dev. Cell* 52:477–91.e8

Randazzo D, Khalique U, Belanto JJ, Kenea A, Talsness DM, et al. 2019. Persistent upregulation of the β-tubulin tubb6, linked to muscle regeneration, is a source of microtubule disorganization in dystrophic muscle. *Hum. Mol. Genet.* 28:1117–35

Raybin D, Flavin M. 1975. An enzyme tyrosylating α-tubulin and its role in microtubule assembly. *Biochem. Biophys. Res. Commun.* 65:1088–95

Redeker V. 2010. Mass spectrometry analysis of C-terminal posttranslational modifications of tubulins. *Methods Cell Biol.* 95:77–103

Redeker V, Levilliers N, Schmitter J, Le Caer J, Rossier J, et al. 1994. Polyglycylation of tubulin: a posttranslational modification in axonemal microtubules. *Science* 266:1688–91

Redeker V, Levilliers N, Vinolo E, Rossier J, Jaillard D, et al. 2005. Mutations of tubulin glycylation sites reveal cross-talk between the C termini of α- and β-tubulin and affect the ciliary matrix in *Tetrahymena*. *J. Biol. Chem.* 280:596–606

Regnard C, Desbruyeres E, Huet JC, Beauvallet C, Pernollet JC, Edde B. 2000. Polyglutamylation of nucleosome assembly proteins. *J. Biol. Chem.* 275:15969–76

Robison P, Caporizzo MA, Ahmadzadeh H, Bogush AI, Chen CY, et al. 2016. Detyrosinated microtubules buckle and bear load in contracting cardiomyocytes. *Science* 352:aaf0659

Rocha C, Papon L, Cacheux W, Marques Sousa P, Lascano V, et al. 2014. Tubulin glycylases are required for primary cilia, control of cell proliferation and tumor development in colon. *EMBO J.* 33:2247–60

Rogowski K, Juge F, Van Dijk J, Wloga D, Strub JM, et al. 2009. Evolutionary divergence of enzymatic mechanisms for posttranslational polyglycylation. *Cell* 137:1076–87

Rogowski K, Van Dijk J, Magiera MM, Bosc C, Deloulme JC, et al. 2010. A family of protein-deglutamylating enzymes associated with neurodegeneration. *Cell* 143:564–78

Roll-Mecak A. 2019. How cells exploit tubulin diversity to build functional cellular microtubule mosaics. *Curr. Opin. Cell Biol.* 56:102–8

Roll-Mecak A. 2020. The tubulin code in microtubule dynamics and information encoding. *Dev. Cell* 54:7–20

Romaniello R, Arrigoni F, Fry AE, Bassi MT, Rees MI, et al. 2018. Tubulin genes and malformations of cortical development. *Eur. J. Med. Genet.* 61:744–54

Saillour Y, Broix L, Bruel-Jungerman E, Lebrun N, Muraca G, et al. 2014. β tubulin isoforms are not interchangeable for rescuing impaired radial migration due to *Tubb3* knockdown. *Hum. Mol. Genet.* 23:1516–26

Sase S, Almad AA, Boecker CA, Guedes-Dias P, Li JJ, et al. 2020. *TUBB4A* mutations result in both glial and neuronal degeneration in an H-ABC leukodystrophy mouse model. *eLife* 9:e52986

Savage C, Hamelin M, Culotti JG, Coulson A, Albertson DG, Chalfie M. 1989. *mec*-7 is a β-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans*. *Genes Dev.* 3:870–81

Schatz PJ, Solomon F, Botstein D. 1986. Genetically essential and nonessential α-tubulin genes specify functionally interchangeable proteins. *Mol. Cell. Biol.* 6:3722–33

Schneider A, Plessmann U, Felleisen R, Weber K. 1998. Posttranslational modifications of trichomonad tubulins; identification of multiple glutamylation sites. *FEBS Lett.* 429:399–402

Schulze E, Kirschner M. 1987. Dynamic and stable populations of microtubules in cells. *J. Cell Biol.* 104:277–88

Schwer HD, Lecine P, Tiwari S, Italiano JE, Hartwig JH, Shivdasani RA. 2001. A lineage-restricted and divergent β-tubulin isoform is essential for the biogenesis, structure and function of blood platelets. *Curr. Biol.* 11:579–86

Sferra A, Fattori F, Rizza T, Flex E, Bellacchio E, et al. 2018. Defective kinesin binding of TUBB2A causes progressive spastic ataxia syndrome resembling sacsinopathy. *Hum. Mol. Genet.* 27:1892–904

Shashi V, Magiera MM, Klein D, Zaki M, Schoch K, et al. 2018. Loss of tubulin deglutamylase CCP1 causes infantile-onset neurodegeneration. *EMBO J.* 37:e100540

Sheffer R, Gur M, Brooks R, Salah S, Daana M, et al. 2019. Biallelic variants in *AGTPBP1*, involved in tubulin deglutamylation, are associated with cerebellar degeneration and motor neuropathy. *Eur. J. Hum. Genet.* 27:1419–26

Shida T, Cueva JG, Xu Z, Goodman MB, Nachury MV. 2010. The major α-tubulin K40 acetyltransferase αTAT1 promotes rapid ciliogenesis and efficient mechanosensation. *PNAS* 107:21517–22

Simons C, Wolf NI, McNeil N, Caldovic L, Devaney JM, et al. 2013. A de novo mutation in the β-tubulin gene *TUBB4A* results in the leukoencephalopathy hypomyelination with atrophy of the basal ganglia and cerebellum. *Am. J. Hum. Genet.* 92:767–73

Sirajuddin M, Rice LM, Vale RD. 2014. Regulation of microtubule motors by tubulin isotypes and post-translational modifications. *Nat. Cell Biol.* 16:335–44

Skultetyova L, Ustinova K, Kutil Z, Novakova Z, Pavlicek J, et al. 2017. Human histone deacetylase 6 shows strong preference for tubulin dimers over assembled microtubules. *Sci. Rep.* 7:11547

Smirnov V, Grunewald O, Muller J, Zeitz C, Obermaier CD, et al. 2021. Novel *TTLL5* variants associated with cone-rod dystrophy and early-onset severe retinal dystrophy. *Int. J. Mol. Sci.* 22:6410

Smith BN, Ticozzi N, Fallini C, Gkazi AS, Topp S, et al. 2014. Exome-wide rare variant analysis identifies *TUBA4A* mutations associated with familial ALS. *Neuron* 84:324–31

Sobierajska K, Ciszewski WM, Wawro ME, Wieczorek-Szukała K, Boncela J, et al. 2019. TUBB4B downregulation is critical for increasing migration of metastatic colon cancer cells. *Cells* 8:810

Soppina V, Herbstman JF, Skiniotis G, Verhey KJ. 2012. Luminal localization of α-tubulin K40 acetylation by cryo-EM analysis of Fab-labeled microtubules. *PLOS ONE* 7:e48204

Strassel C, Magiera MM, Dupuis A, Batzenschlager M, Hovasse A, et al. 2019. An essential role for α_{4A}-tubulin in platelet biogenesis. *Life Sci. Alliance* 2:e201900309

Sun X, Park JH, Gumerson J, Wu Z, Swaroop A, et al. 2016. Loss of RPGR glutamylation underlies the pathogenic mechanism of retinal dystrophy caused by TTLL5 mutations. *PNAS* 113:E2925–34

Suryavanshi S, Edde B, Fox LA, Guerrero S, Hard R, et al. 2010. Tubulin glutamylation regulates ciliary motility by altering inner dynein arm activity. *Curr. Biol.* 20:435–40

Szczesna E, Zehr EA, Cummings SW, Szyk A, Mahalingan KK, et al. 2022. Combinatorial and antagonistic effects of tubulin glutamylation and glycylation on katanin microtubule severing. *Dev. Cell* 57:2497–13.e6

Szyk A, Deaconescu AM, Piszczek G, Roll-Mecak A. 2011. Tubulin tyrosine ligase structure reveals adaptation of an ancient fold to bind and modify tubulin. *Nat. Struct. Mol. Biol.* 18:1250–58

Szyk A, Deaconescu AM, Spector J, Goodman B, Valenstein ML, et al. 2014. Molecular basis for age-dependent microtubule acetylation by tubulin acetyltransferase. *Cell* 157:1405–15

Tang Q, Liu M, Liu Y, Hwang RD, Zhang T, Wang J. 2021. NDST3 deacetylates α-tubulin and suppresses V-ATPase assembly and lysosomal acidification. *EMBO J.* 40:e107204

Tas RP, Chazeau A, Cloin BMC, Lambers MLA, Hoogenraad CC, Kapitein LC. 2017. Differentiation between oppositely oriented microtubules controls polarized neuronal transport. *Neuron* 96:1264–71.e5

Ten Martin D, Jardin N, Giudicelli F, Gasmi L, Vougny J, et al. 2022. A key role for p60-Katanin in axon navigation is conditioned by the tubulin polyglutamylase TTLL6. bioRxiv 2022.01.20.477127. **https://doi.org/10.1101/2022.01.20.477127**

Thazhath R, Liu C, Gaertig J. 2002. Polyglycylation domain of β-tubulin maintains axonemal architecture and affects cytokinesis in *Tetrahymena*. *Nat. Cell Biol.* 4:256–59

Ti SC, Alushin GM, Kapoor TM. 2018. Human β-tubulin isotypes can regulate microtubule protofilament number and stability. *Dev. Cell* 47:175–90.e5

Tian G, Cowan NJ. 2013. Tubulin-specific chaperones components of a molecular machine that assembles the α/β heterodimer. *Methods Cell Biol.* 115:155–71

Tischfield MA, Baris HN, Wu C, Rudolph G, Maldergem LV, et al. 2010. Human *TUBB3* mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *Cell* 140:74–87

Torrino S, Grasset EM, Audebert S, Belhadj I, Lacoux C, et al. 2021. Mechano-induced cell metabolism promotes microtubule glutamylation to force metastasis. *Cell Metab.* 33:1342–57.e10

Tran AD, Marmo TP, Salam AA, Che S, Finkelstein E, et al. 2007. HDAC6 deacetylation of tubulin modulates dynamics of cellular adhesions. *J. Cell Sci.* 120:1469–79

Valenstein ML, Roll-Mecak A. 2016. Graded control of microtubule severing by tubulin glutamylation. *Cell* 164:911–21

Van Dijk J, Rogowski K, Miro J, Lacroix B, Edde B, Janke C. 2007. A targeted multienzyme mechanism for selective microtubule polyglutamylation. *Mol. Cell* 26:437–48

Velle KB, Kennard AS, Trupinic M, Ivec A, Swafford AJM, et al. 2022. *Naegleria*'s mitotic spindles are built from unique tubulins and highlight core spindle features. *Curr. Biol.* 32:1247–61.e6

Vemu A, Atherton J, Spector JO, Moores CA, Roll-Mecak A. 2017. Tubulin isoform composition tunes microtubule dynamics. *Mol. Biol. Cell* 28:3564–72

Vemu A, Atherton J, Spector JO, Szyk A, Moores CA, Roll-Mecak A. 2016. Structure and dynamics of single-isoform recombinant neuronal human tubulin. *J. Biol. Chem.* 291:12907–15

Vemu A, Garnham CP, Lee D-Y, Roll-Mecak A. 2014. Generation of differentially modified microtubules using in vitro enzymatic approaches. *Methods Enzymol.* 540:149–66

Vent J, Wyatt TA, Smith DD, Banerjee A, Ludueña RF, et al. 2005. Direct involvement of the isotype-specific C-terminus of β tubulin in ciliary beating. *J. Cell Sci.* 118:4333–41

Verdier-Pinard P, Wang F, Burd B, Angeletti RH, Horwitz SB, Orr GA. 2003. Direct analysis of tubulin expression in cancer cell lines by electrospray ionization mass spectrometry. *Biochemistry* 42:12019–27

Wall KP, Hart H, Lee T, Page C, Hawkins TL, Hough LE. 2020. C-terminal tail polyglycylation and polyglutamylation alter microtubule mechanical properties. *Biophys. J.* 119:2219–30

Wall KP, Pagratis M, Armstrong G, Balsbaugh JL, Verbeke E, et al. 2016. Molecular determinants of tubulin's C-terminal tail conformational ensemble. *ACS Chem. Biol.* 11:2981–90

Wang D, Villasante A, Lewis SA, Cowan NJ. 1986. The mammalian β-tubulin repertoire: hematopoietic expression of a novel, heterologous β-tubulin isotype. *J. Cell Biol.* 103:1903–10

Wang N, Bosc C, Ryul Choi S, Boulan B, Peris L, et al. 2019. Structural basis of tubulin detyrosination by the vasohibin-SVBP enzyme complex. *Nat. Struct. Mol. Biol.* 26:571–82

Wang X, Fu Y, Beatty WL, Ma M, Brown A, et al. 2021. Cryo-EM structure of cortical microtubules from human parasite *Toxoplasma gondii* identifies their microtubule inner proteins. *Nat. Commun.* 12:3065

Webster DR, Gundersen GG, Bulinski JC, Borisy GG. 1987. Differential turnover of tyrosinated and detyrosinated microtubules. *PNAS* 84:9040–44

Weisbrich A, Honnappa S, Jaussi R, Okhrimenko O, Frey D, et al. 2007. Structure-function relationship of CAP-Gly domains. *Nat. Struct. Mol. Biol.* 14:959–67

Widlund PO, Podolski M, Reber S, Alper J, Storch M, et al. 2012. One-step purification of assembly-competent tubulin from diverse eukaryotic sources. *Mol. Biol. Cell* 23:4393–401

Wloga D, Rogowski K, Sharma N, Van Dijk J, Janke C, et al. 2008. Glutamylation on α-tubulin is not essential but affects the assembly and functions of a subset of microtubules in *Tetrahymena thermophila*. *Eukaryot. Cell* 7:1362–72

Xia L, Hai B, Gao Y, Burnette D, Thazhath R, et al. 2000. Polyglycylation of tubulin is essential and affects cell motility and division in *Tetrahymena thermophila*. *J. Cell Biol.* 149:1097–106

Yadav A, Matson KJE, Li L, Hua I, Petrescu J, et al. 2023. A cellular taxonomy of the adult human spinal cord. *Neuron* 111:328–44.e7

Yen TJ, Machlin PS, Cleveland DW. 1988. Autoregulated instability of β-tubulin mRNAs by recognition of the nascent amino terminus of β-tubulin. *Nature* 334:580–85

Zadra I, Jimenez-Delgado S, Anglada-Girotto M, Segura-Morales C, Compton ZJ, et al. 2022. Chromosome segregation fidelity requires microtubule polyglutamylation by the cancer downregulated enzyme TTLL11. *Nat. Commun.* 13:7147

Zhang X, Yuan Z, Zhang Y, Yong S, Salas-Burgos A, et al. 2007. HDAC6 modulates cell motility by altering the acetylation level of cortactin. *Mol. Cell* 27:197–213

Zheng P, Obara CJ, Szczesna E, Nixon-Abell J, Mahalingan KK, et al. 2022. ER proteins decipher the tubulin code to regulate organelle distribution. *Nature* 601:132–38

Zivraj KH, Tung YCL, Piper M, Gumy L, Fawcett JW, et al. 2010. Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs. *J. Neurosci.* 30:15464–78